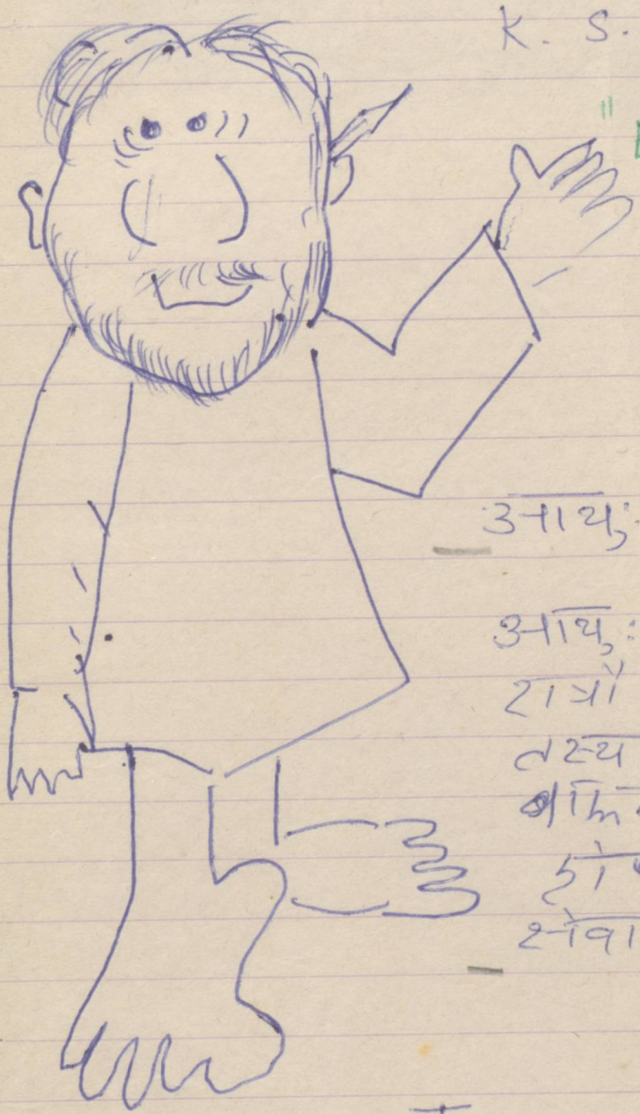
RELATION BETWEEN TRANSMITTANCE (T) & OPTICAL DENSITY (O.D)

100	0.000	75	0.125	50	0.301	25	0.602
	002		128		305		611
99	0.004	74	0.131	49	0.310	24	0.620
	007		134		314		629
98	0.009	73	0.137	48	0.319	23	0.638
	011		140		324		648
97	0.013	72	0.143	47	0.328	22	0.658
	015		146		332		668
96	0.018	71	0.149	46	0.337	21	0.678
	020		152		342		688
95	0.022	70	0.155	45	0.347	20	0.699
	025		158		352		710
94	0.027	69	0.161	44	0.357	19	0.721
	030		164		362		733
93	0.032	68	0.168	43	0.367	18	0.745
	034		171		372		757
92	0.036	67	0.174	42	0.377	17	0.770
	039		176		382		783
91	0.041	66	0.181	41	0.387	16	0.796
	044		184		392		810
90	0.046	65	0.187	40	0.398	15	0.824
	049		190		404		839
89	0.051	64	0.194	39	0.409	14	0.854
	053		197		414		870
88	0.056	63	0.201	38	0.420	13	0.886
	059		205		426		904
87	0.061	62	0.208	37	0.432	12	0.921
	063		212		438		940
86	0.066	61	0.215	36	0.444	11	0.959
	068		219		450		979
85	0.071	60	0.222	35	0.456	10	1.000
	074		226		463		023
84	0.076	59	0.229	34	0.469	9	1.046
	079		233		475		071
83	0.081	58	0.237	33	0.482	8	1.007
	084		241		489		126
82	0.086	57	0.244	32	0.495	7	1.155
	089		248		502		186
81	0.092	56	0.252	31	0.509	6	1.222
	095		256		516		261
80	0.097	55	0.260	30	0.523	5	1.301
	100		264		530		349
79	0.102	54	0.268	29	0.538	4	1.398
	105		272		545		
78	0.108	53	0.276	28	0.552	3	1.523
	111		280		560		1.611
77	0.114	52	0.284	27	0.569	2	1.699
	117		288		571		1.850
76	0.119	51	0.291	26	0.585	1	3.000
	122		296		593		

O.D. = 2 - log T



K. S. KRISHNAN

MBV IISc 560012

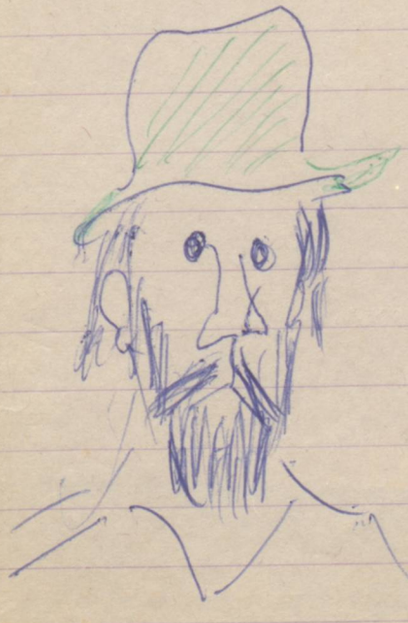
"EVEN I DON'T (RATHER CAN'T) READ THEM, WHY DO YOU BOTHER'

Record 1|3|74 –

आयुः परिस्रवति भिन्नघटादिवांभः

आयुः वर्षशतं शुचि वृणां परिमितं
रात्रौ तदर्धं गतं
तस्यार्धस्य परस्य चार्धमपरं
व्याधि त्वदुत्सवयोः
शेषं व्याल वियोगदुःख सहितं
सेवादिभिर्नीयते

TIME WOUNDS ALL HEALS



WHY TELL THE TRUTH
WHEN A LIE
COULD DO AS WEL

BEGGARS
CANT BE
CHOOSERS

51
100
102
8

52
100
1380 1480
12

53
100
5·35 74 (4b) 174
9

#① 520ng/ml
54
100

9.8 135 (30) 234

17

#[illegible]

55

100

5.65 78 (42) 178

12

#② 560ng/ml

56

100

8.4 116 (35) 216

9

~~#③~~

57

100

6.35 60 (50) 160

14

③ 510 ng/ml 58

100

5.8 80 (42) 180

9

59

100

2.4 32 (58) 132

19

④ 510 ng/ml 60

100

13.8 100 (22) 204

10

61

100

6.3 87 (42) 187
9

#⑤ 560 ng/ml 62
100
8.4 116 (35) 216
9

63
100
6.1 84 (42) 184
18

#⑥ 630 ng/ml 64
100
5.9 82 (42) 182
15

65
100
6.9 95 (39) 195
10

#⑦ 415 ng/ml 66
100
13.5 185 (22) 285
13

67
100
7.6 105 (36) 205
15

#⑧ 370 ng/ml 68
100

15.8 219 (19) 319
6

69
100
29 391 496
79 (7) 15
#⑨ 70 ng/ml 70
72 100
3 511 (50) 611
(3.7) 11

157

A A	Sample III	IV
Cys Acid	1.107	1.687
Asp	7.995	8.896
Thr	3.101	2.861
Ser	2.032	1.469
Glu	11.47	10.91
Pro	4.665	4.812
Gly	5.003	7.034
Ala	10.53	7.603
Val	5.316	5.927
Cys2	— NIL —	— NIL.
Met	0.3884	0.5640
Ile	5.299	5.728
Leu	9.423	9.399
Tyr	2.168	2.319
Phe	3.383	3.450
Lys	6.4	5.406
His	1.92	2.074
Amm	15.37	15.33
Arg	4.394	4.835

Lost

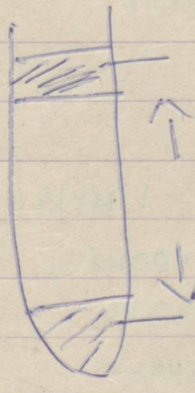
Monkey 'B' 40/28

1-3-74

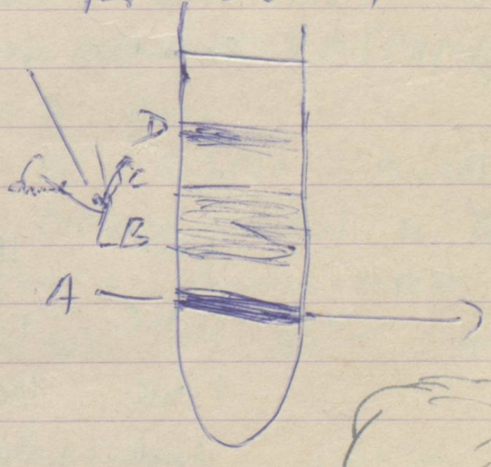
Adult Macacus Radiata - Mg Soy paralysis
Bled by cardiac. Brain kept in freezer

2.3.74

Shaved 4 cervical region - top frey portion
Tissue. 4 brains homogenised in
Dounce (Self made) with hand 20 strokes.
3000 rpm 30 sec pellet discarded
Supt - 10,000 rpm 10 min
pellet - white portion is discarded in
hand — Dirty bottom blackish material
and to go in Isopropanol.
The discarded-up material homogenised
first in gradient as shown



Spun for 30 min at 27000 rpm
(≈ 65000 g)



3.3.74

NMR Studies

2.5 mg A. Choline in 1 ml D_2O

ATP: 551
AMP .369

ATP

1 ml	1 M	551 mg
1 ml	.00125	$\frac{551}{800}$ mg.
4 ml	.00125	2.76 mg
2 ml	.00125	1.38
2.5 ml	.00125	1.73 mg.

AMP

1 ml	1M	369 mg
1 ml	1 mM	.369 mg
1 ml	100 μM	.0369 mg
1 ml	500 μM	.1945
5 ml	"	1.1680

① pure A chrome spectrum.
② Added old RB. membs in D_2O. (= 2ml)
③ further 25/2/74 MB freeze dried preparation — change observed (≈ 3mg)
④ A chrome + 2/3/74 preparation unchalysed 2ml).

D_2O old RB. preparation
⑤ Acetone extract in $CDCl_3$.
⑥ $CHCl_3$: MeOH : H_2O. extract in $CDCl_3$.

4-3-74 Electron microscopy undone.

Further nmr of ③ & ④ kept in fridge labelled I & II.

5.3.74.

Nat · kt ATPase. of 2/3/74

Rks.

a. Nacl. 0.25 M $MgSO_4$ 0.00138 M
b. Nacl 0.25 M $MgSO_4$ 0.00138M kcl 0.0125M
c. Na ATP 0.00125 M Freshly prepared
d. Tris Hcl 0.0625 M (PH 8.6) Na EDTA 3.12×10^{-4} M
e. TCA 30%.

(Na^+) 0.4 ml of (a) & (c) mixed with 0.16 ml D
Mix x more chilled membs added in 0.04 ml (chloride conductivity serves $Na^+ + H^+$) identical except b in place of a (5-50 µg protein)
Incubate for 30 mts at 37°C terminate by 0.3 ml 30% TCA.

s' nucleotidase 0.05 M Glycine NaOH (9.1 pH)
1 ml buffer + 50 µM AMP + 1 ml $MgCl_2$ + 0.2 ml Membs (50 µg protein)
Incubate 15 mts terminate by 2.5 ml TCA 5%.

Glaser Finske - Subbarow method.

5' Nucleotidase

1. Blank. 0
2. H.C (crude) (24) 0.155 // →
3. PND. (.02) 0.12
4. Pellet (10,000 CFU media) 0.1
5. A. (Mem b) 0.125 // →
6. B. (My.) 0.05

15'

NaT ICT ATPase

(Reaction process Glormula)

1 = OD .623 µM

Blank 7 0

AC 8 9 (10⁻⁴) ≫ Na le

30' PND 10 No Expt 11 Excellent 0.17 0.22

Prop,m 12 No Expt 13 excellent

A 14 15 (10⁻⁴) ≫ 0.175 0.23

B. 16 No Expt 17 excellent

Standard.

~~0.50~~ 100 = 0.66 µM.

Protein

1	Blank	.02	0
2	RBS µ (ORD)	.02	51
3	MBSM (ORD)	.07	36
4	HC	.02	180
5	PND	.01	264
6	Pellet (10,000)	.101	206
7	A Membs	.102	160
8	B (My)	.02	67

Standard.

Take previous week standard in expt 7.

problem:

DSA

0 0

60 Mg/ml 94

120 µg/ml 186

160 µg 246

6.3.74

NMR tubes ① & ② followed again

6.3.74 8.30 PM I / 2.5 mg Acetone in 1 ml D_2O
tube I

6.3.74 tube II 0.5 mg/ml Acetone in D_2O (twice diluted)

tube III 2.5 mg/ml Acetone + Killed Mem (Sonicated) ≈ 3 mg

~~tube IV~~ IV Acetone extract in $CDCl_3$.
Sonicated mem in D_2O VI
Protein residue dil. NaOD aliquots VII

Tube V Sucrose 20 mg. in D_2O

< Red ① Acetone + Mem — VIII
< Red ② Acetone + Mem IX

VI Sonicated mem in D_2O
(Pre freeze dried)

VII Protein Residue in D_2O
~~extract~~

[I + with 0.25 ml of VI .. special tube
Marcel 8 74]

BKM ↓ Date ↓ month ↓ year (last digit) ↓ 000 Number

Sample BKM 834 001
002 etc
Number

Sample No. 1 + B
RB
Ach
etc

Dry hd

6-3-74

Fluorescence. Dry 7.4

Excitation 340 mμ
Emission 480 mμ

I	II	III	IV	
Blank + (A, NOVS)	Native + (Buffer)	Native + Acr	Native + NOVS	
5 A	51	48	50.5 N	0
5 N	47.5	46.5	49.5 N	+ .01
8 N	50	49	55 N	+ .02
8 A	47	48	55 N	+ .05
8 N	45.5	50	56 N	+ 0.1
8.5 N	40	48	Above 100 in 1 ml (120) = 30 in 1/10 rxn	+ 0.2
8.5 N	38	45	= 54 in x 1/10 rxn	+ 0.2
9 (S)	38 (Buff)	45.5 (+S)	Do (repl)	+ .05
9.5 (S)	41	51 (+S)	Do	+ 0.05
10.5	39	43	Do	Lyoph in between

Our Centrifuge
~~Remi~~
T-23
Remi

Rio Marker brain - fresh perhaps - Extracts fully 40 - 25 %. Intact phos fully Collected - Dialysis in 40 hrs and freeze dried & lipid extracts. Not in Cold

Freeze dried preparation
Chloroform - Methanol mixture
7/8 3/74 approx 9 : 10 : 10 ratio

(1) Settled mitom Centrifuged pellet - dried & Reprocessed

(2) The lipid Centrifuge supt - vacuum dried & lipids kept. in future Expts

8/3/74 NMR - By Simon on left

9/3/74 / 10/3/74 Invitro morning

11-3-74 Fresh monkey brains - washed in Saline & bufr (Tris 7.4 10mM) - Grey matter melted Cerebellar - minced (75 gms approx) and homogenised in buffer only (1+C (made homogenate)

Rem in RC2B Sorvall 3000 rpm 10 min -

- Pellet like nuclei & Debris - PND.

Supt spun at 6000 rpm 5 mts

Pellet - dissolved by (6000 pellet + 6000 supernatant) Tris & put on gradient

40 - 28 %. 30 mls 64000 g (I st mt)

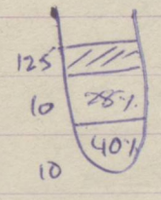
mitochon collected (40% I st mt) (I st B top of 28%) dissolved twice Tris and again put on 40-28% gradient.

II nd A below 40% Mitochon (II nd B mt) + (II nd B top of 28%).

Dialyse all nt 20 hrs. [Then enzyme assays. & protein assays. Have Standards.]

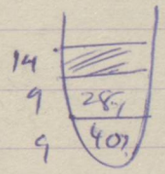
Knots

	B	S	
HC	1	1	No weights
PND	2	1	
Spt 6000	3	1	
Pellet 6000	4	2	
I A	2	2	
I B	3	2	
I C	1	3	
II A	2	3	
II B	00	3 (Two bags)	
II C	4	1	
II D	4	2 (2 small bags)	
JUNK	4	3	

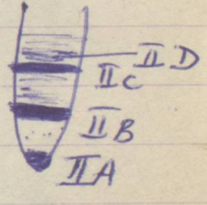


24000 (6 hours)
30 mls

dissolved twice by bufr



24000 (6 hours)
30 mls



[Junk dissolve thin fm all over]

c. 6.92 mg 10 ml · 0.00125 M ATP

1.384

~~8.3~~ Tris 1N. 100 ml 12.11 gms //

(Dissolve in 50-60 ml - Hcl adjust
PH 8.5 finally make upto 100 ml)

Na EDTA (BDH) 372.25

100 ml 1M 37.225

100 ml 3.12 mM $\frac{37.225 \times 3.12}{1000}$ gm.

74450
37225
101675
1051.6200

1.051 gms. (Dilute 100 times for use)

Assays

I Na⁺ K⁺ ATPase.

Reagents

A: NaCl 0.25 M, $MgSO_4$ 0.00138 M.

B. NaCl 0.25 M, $MgSO_4$ 0.00138 M KCl 0.0125 M

Freshly prepared > C. Na ATP 0.00125 M

D. Tris HCl 0.0625 M (PH 8.6; Na EDTA 3.12×10^{-4} M)

E. TCA 30%.

Procedure:

Wallach & Kamin
Meth Enzym VIII 164-172

Series Na⁺. 0.4 ml each of A & C mixed with 0.16 ml of D. mixture chilled. Membrane added by 0.04 to make final volume 1 ml.

Series Na⁺ + K⁺ identical except B instead of A

Incubate for 30 mts (or 60 mts) at 37°C

Terminate by 0.3 ml TCA (30%). Centrifuge at 10,000 rpm. for 5 mts 0-4°C

Inorganic phosphate in 1 ml aliquot of supt by Fiske-Subbarow method. Develop Colour at 0°C

Activity μMoles P_i/mg protein/hr.

II 5' Nucleotidase L.A. Heppel & K.J Helmore
Meth in Enzymol Vol II 546-

Reagents

A. 1.0 M glycine-NaOH buffer PH 8.5

B. 0.1 M $MgCl_2$

Fresh (in cold) C. 5'-AMP solution

Prepared D. Enzyme (or Membr).
(kept frozen)

A total volume of incubating mixture of 1.24 ml is made to contain 0.1 ml of buffer (A) 0.1 ml of (B) 3 micromoles (C) & enzyme. Incubation is for 15 mts at 37°C & reaction

$$\frac{\text{Change in OD} \times 10^6 \times 60}{6.22 \times 10^3 \times \text{mg Tissue} \times \frac{1}{1000} \times \frac{.1}{1.1}}$$

μ Moles / mg Tissue / hr

Range 60

60 μ moles / mg tissue / hr

A: 19 mg / 10 ml neutralized Solution

.1 M : 5.5 gm / 100 ml

1.1 dil x 20 ml

Alkaline Solution 19 mg / 2 ml

Freshly neutralize & make up to 10 ml

.1 ml for every assay.

Stopped by addition of TCA in final concentration of 5%. If necessary the mixture is centrifuged. Phosphate by Fiske-Subbarow. Usually supernatant clear & necessary

µMole/mg protein/hr

III NADH diaphorase: [Endoplasmic reticulum marker]

(Reference above possible)

A. 0.02 M Tris Hcl (PH 7.4)

B. 0.003 M Pot Ferricyanide

C. NADH (DPNH) 2 mg in 10 ml

Oxidation of NADH measured spectrophotometrically at 20°C by decrease in absorbance at 340 mµ in a Quartz cuvette the following are pipetted:

0.4 ml of A. 0.6 ml of C

and 0.2 ml of ferricyanide (B)

To this 0.05 ml of enzyme added (40-20 µg protein). The solution mixed by inverting and readings taken at 15 second interval for 2-5 min.

Decrease in optical density. Linear function of time up to 80% oxidation.

The extinction coeff. which is 6.22×10^3 liter mol^{-1} cm^{-1}

Activity µM of NADH / mg protein / minute.

W D Bonner

SDH: Mitochondrial Enzymes Meth Enzym I 722

A ~~0.1 ml~~ 0.1 ml Pot Cyanide (neutralized) KCN. (+HCl to neutralize)

B. 0.01 M $K_3Fe(CN)_6$

C 0.2 M Na Succinate in suitable buffer (PH 7.2) (Phosphate & Tris)

3 Gms Sod bisulfate in 2 oz water.

0.1 ml of A.

To a 1 cm cell add 0.3 ml ~~each~~ of ~~A~~, B and 0.2 ml of C. Buffer added to give final ~~gms~~ ~~qty~~ ~~volume~~ of 3.0 ml. Water only in reference cell. At zero time 0.2 ml of tissue diluted preparation added to both cells & optical ~~activity~~ density at 400 mμ followed as a function of time. At close of each determination temperature recorded.

The activity of system which reduces $K_3Fe(CN)_6$ expressing decrease of OD at 20°C

Fiske Subbarow

A. 10 N H_2SO_4 30 ml.
2.5 gms Ammm Molybdate 20 ml H_2O
Make up with washings to 100 ml.

B. 19.5 ml 15% Sod bisulfite.
40 mg ANSA
0.5 ml Sod Sulfite (20%)
Add more Sod Sulfite if necessary; keep cold not more than 6 weeks.

Assay in Aliquots of 1 ml. Volume.
0.5 ml A. 0.2 ml B.
Make up with water to 10 ml.

Dreilen - Lowry etal.

A I 2% Na_2CO_3 in 0.1 N NaOH.
A II 1% $CuSO_4$.
A III 2% Sod-pot tartarate
stable

50 ml A I 0.5 ml A II 0.5 ml A III mixed up at time of assay
Add 5 ml to 1 ml aliquot
0.5 ml Folin's (diluted reagent) added after 10 mts.

Dilute Refrigerate

SRK'S

SDH

O D/anle × 1000 / 5.41 / mg protein

millimole

pmole per reduced / min / mg prot

13/3/74

Protein

1.	Blank		0
2.	BSA 0.2 ml	(300 µg/ml)	94
3.	BSA 0.4 ml	(300 µg/ml)	186
4.	BSA 0.6 ml	(300 µg/ml)	246
5.	0.02	H.C	720
6.	0.02	PNP	>1000
7	0.01	6000 pellet	650
8.	0.02	6000 Supt.	405
9	.02	IA	
10	.02	IB	155
11	.02	IC	86
12	.02	II A	220
13	.02	II B	252
14	.02	II C	91
15	.02	II D	85
16	.02	JUNC	37

to repeat of the dilution

today Repeat certain dominant protein to run & do.

keep readings below 200

0 18 ml II B for drying.

① 5 ml IC + 5 ml II C + 2 ml II D dried.

ATP 551 8.304 mg / 12 ml 0.00125 ATP

12.1 gm Tris in 100 ml D.D pharmlee

Amp. 369

20 tubes 10 μM

369 mg. 1000 ml. 1 mM

3.69 mg 10 ml 1 mM

.369 mg 1 ml 1 mM

1.845 mg 10 ml 0.5 mM

1.845 mg 1 ml 5 mM

(Dilute 100 Tris in tube)

.04 g AMP : 50 μM

.04 200 μM

① .03 150 μM

② .01 g enzyme preparation.

.1 ml A .1 ml B 1 ml H_2O.

Incubate 15 min

Terminate .2 ml 30% TCA

1M Live Dilute 1 ml

1 mM 1 ml.

100 μM .1 ml

1M live 1 ml

100 μM 1 ml

.05 50 μM

25 μM .83 ml

13/3/74

Na⁺ K⁺ ATPase · ·4 A a·4D +·4C +·16 D
cardiac

Membr added as above.

			colours/tubes	.2 ml aliquots
Hc .01	1A	2A	19	33
PNP .01	3A	4A	37	27 ?
boco pellet .01	5A	6A	55	46 ?
boco Spl .02	7A	8A	38	23 ?
1A .02	9A	10A	23	28
1B .02	11A	12A	20	33
1C .02	13A	14A		
II A .02	15A	16A		
II B .02	17A	18A	15	24
II C .02	19A	20A		
Stds	21A	22A	0.0	0.10
	23A	24A	.05	0.15

(.01 ml · 1 µM.)

(total 2 ml · 1·2 ml · 1·2 ml · 50 1/6 ml P₁)

30 mls

21A 0
22A 256
23A 460 (max .01 make stock)
24A 740

5' nucleotidase

Hc .01	1	0	13
PNP .01	2	0.05	14
boco pellet .01	3	0.10	15
boco Spl .02	4	0.15	16
1A .02	5		
1B .02	6		
1C .02	7		
II A .02	8		
II B .02	9		
II C .02	10		
II D .02	11		
Joints .02	12		

Stds

·1 ml A
·1 ml B
1 ml H_2O
·03 AMP

(brown Conc. 7 AMP × Stock Conc. × Stock vol. per tube)

Repeat tomorrow

(Colour fount - many by blank)

Centrifuged all and phosphate estimation in 0.2 ml aliquots (Cleans too faint

---

Can go upto 0.5 ml ~~of buffer~~ of buffer but full colour not seen centrifuging.

~~Phosphate~~ Acts etc

* Also phosphate std Max 1 mM
  (marked by 21A) actual 101 7 spots
* Maximum

14/3/74

M : Sonicated
35 mg dried membrane in D_2O (1 ml)

For ORD Experiment

2.5 ml of original IIB made to
5 ml Stock 2B → Sonicated ½' 6A → S2B

S2B — 100 dilution ORD taken
(Cannot do Lowry as more next time)

S2B – Stock 2B Sonicated ½ minute 6 Amps

15/3/74

Monkey brain in 10% Sucrose Stored at 0°
(~~ice pack~~ in ice all freq.).

16/3/74 24hrs Stored brain - Monkey Macacus Radiata
Grey portions (Cerebellum absent) Sliced -
Dounce homogenisation 20 Strokes without foaming
by hand in 10% Sucrose in Tris Hcl 7.4 10mM
buffer. - Total buffered Sucrose approx 4 times tissue
volume.

Note variation

Centrifuged at 4000 rpm, Serial 4.25 rotor. 2x 20ml
Supernatant + fluffy top portion
poured to other centrifuge tubes
Kept rest at 10,000 rpm 10mins
Pellet diluted by 10% buffered sucrose & kept
volume.
Layered over 40-28% discontinuous Sucrose gradient
Spun at 13000 rpm (20000g)
for 20mins.
Soluphase + layered out
diluted with Tris Hcl 10mM buffer to
about 3 times original volume.
This kept at two depths.

Monkey brain Grey portions sliced
↓
Homogenised in 10% Sucrose in Tris 10mM

Stored for full 48hrs
and thin
Layered over 40/28%.

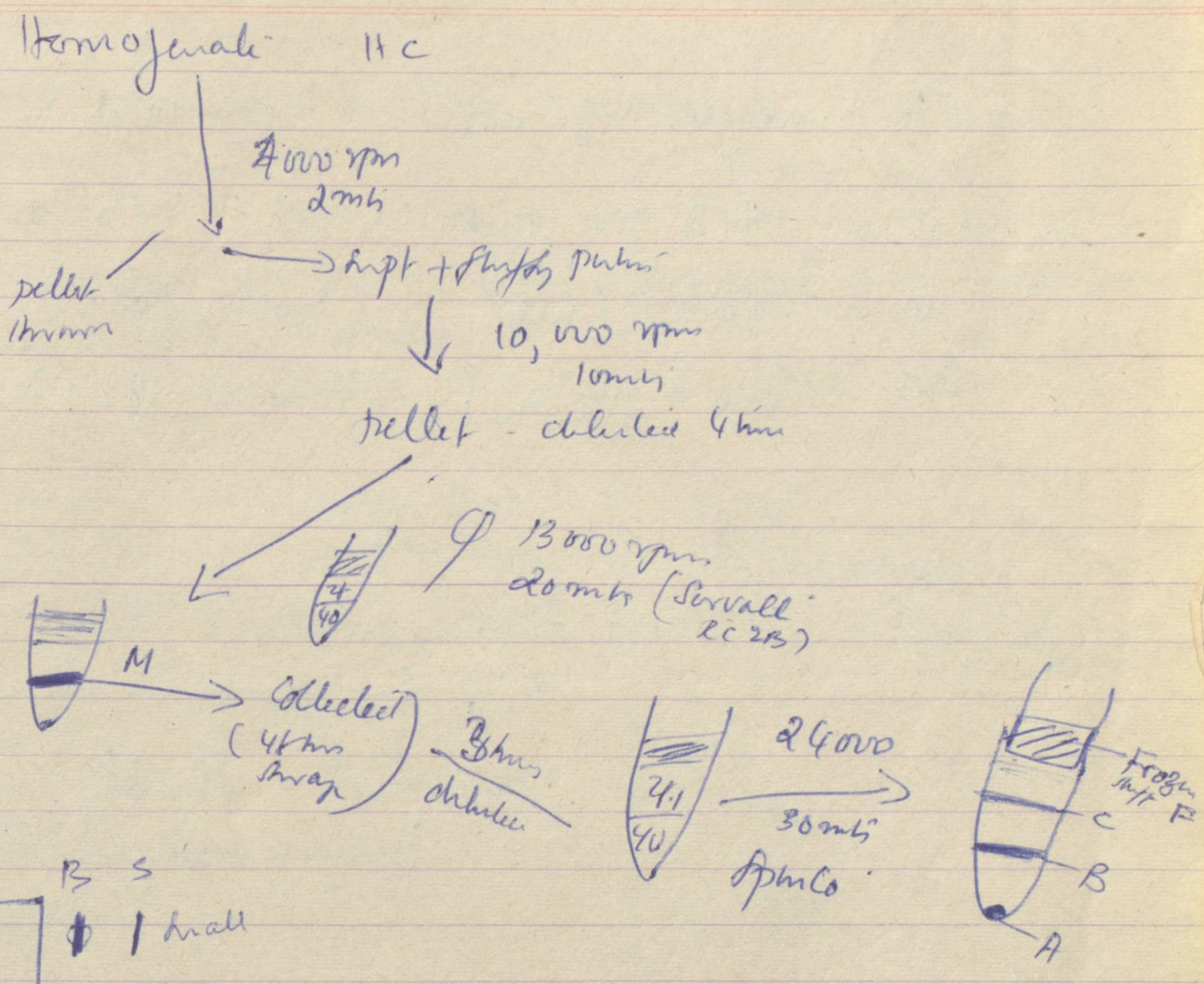
16/3/74 Sucrose fraction and soluphase collected
Dialysed against DDW for 24hrs.

Gly
Dispa.
Glycine

Add HCl small

Buffers

	pH	7.4	5.00	3.00
I	Blank → Conc of	Ach, Serotin, NA	GABA	.5 M, 50 mM.
II	With Methods (.5 ml to 10 ml. 2 buffers)	→		(2 M → 5 1/4 g Acetate in 1/10 → Measure)
III	Methods in H2O Sucrose/Calcium		+ Add to make 1 M	
IV	NaOD Methods			
V	DNS	Small 1 ml 10 ml also pH.		
		Has Sucrose methods in buffer.		



	B	S	
HC			small
M	00		① Estimate protein by Lowry
A	21		
B	01	red	② Enzymes. SDH & NADH diaphorase
C	22		ATPase nucleotidase
F	12		③ Fluorescence - at different pH's.
			④ ORD / Cass in 10 mM Acetate.
			⑤ Freeze dry left over 6 mg.

18/3/74 5.30 daily Starch 2 hours DPW
1st Watch change 10:30 PM

N	8.4 mg / ml	(Mol wt 168.2) ≈ .05M
S	19.3 mg / ml	(Mol wt 386.3) ≈ .05M
G	22 mg / ml	(Mol wt 103.) ≈ 0.2M
A	36.3 mg / ml	(Mol wt 181.2) ≈ 0.2M

N: Not dissolve in alcohol -
the fate powder in water
- After some time slightly
brownish solution (Flesh
colour)

18/3/74

1. DB.

Sonicated Marker from Membrane in D_2O

PH = 6.2.

(Put 29.2 mg Nacl to make 1M.

(1134 DM Nacl) Accepted value)

2. Protein residue

in D_2O . Obtd. 25/2/74 Accept PH . 14.

PH values	Buffer. 1.5 ml	Buffer. 9 ml + 1 ml membs 1.5 ml	Water 4.5 ml + 0.5 ml membs 1.5 ml	Acetyl choline Quantity added
	7.4 - 7.5	7.4 - 7.5	5.3	0
	7.5	7.4	5.3	.01
	7.5	~	5.2	
	7.4 - 7.5	7.3 - 7.4	5 - 5.1	+ .04
				+ 0.2
				~~+0.5~~
	7.5	7.4	5.1	N
	7.5	do	6.	0
	7.5 - 7.6	do	7.8	+ '01
	7.6 - 7.7	7.4 - 7.5	8.4	+ 0.04
				+ 0.2
				~~+0.5~~
	7.5	7.4	5.5	S 0
	7.4 - 7.5	7.2	3.4	.01
	7.3 - 7.4	4.0	2.8	+ 0.04
	~		2.5	+ 0.2
				~~+0.5~~

Buffer	Buffer + mucin	Gelatin + mucin	Cr
7.3-7.4	7.2-7.3	5.3	0
7.3		5.5	+0.01
7.3-7.4	7.2	5.8	+0.04
7.3-7.4		6.1	+0.2

TNS

~	7.3 7.2-7.3	~	1.35 ml + 300 mg/ml soln to 3.5 ml (mucin + buffer)

19/3/74 (6/3/74 Rough preparation)

$CHCl_3$: meo : gelatin lipid extract

Lyophilized.

Added 20 ml $CHCl_3$ -

$CHCl_3$ layer separated and added

10 gm Sodium sulphate anhydrous -

Clear dried lipid extract kept

in ambient temp

Label 634 lipid

11/3/74. Membrane (II B) Lacrimal

& Combined (I C II C II D) Freeze dried

Upcoming lab: Calc NMR & IR w chlorofm

Hydrophonic bands broken by HNO3 acid treatment

II B $CHCl_3$: MeOH (2:1) Lipid Extract — 1134 II BL Label.

CD extract $CHCl_3$: MeOH (2:1) Lipids Label 1134 CDL

Dried with Na Sulphate is kept highly Evacuated in cold.

All Applied in TLC.

Spots

① Lipids G3A stuff — Extreme right two points

② Lipids 1134 II BL — Middle two points

③ Lipids 1134 CL — Extreme left two points

④

66.12

MB/24

Na Succinate.

NADH

ATP

DMP: 3 µM. in 1.25 ml

1 ml. of 1M Soln — 1 mM.

1 ml of 1 mM Soln — 1 µM.

1 ml of 10 mM — 1 µM

1 ml of 30 mM — 3 µM.

3.69 mg/ml = 10 mM

11.07 mg/ml 30 mM.

11.07 mg in 1 ml

② ATP 6.61 mg/10 ml ATP Soln

③ NADH 1 mg in 5 ml

4. Succinate 0.2 M.

① Nat Ic+ ATPase .02 ml membrane in all except when stated

.3 ml 30% TCA

② 5' nucleotidase .02 ml membrane in all except when stated

.1 ml - 5 ~~2.63~~ 11.07 mg/ml BMP = 3 µM in assay

. Total assay is 1.22 ml

Terminate
2.5 ml 8% TCA

TLC

Blank in Lecture

I & II

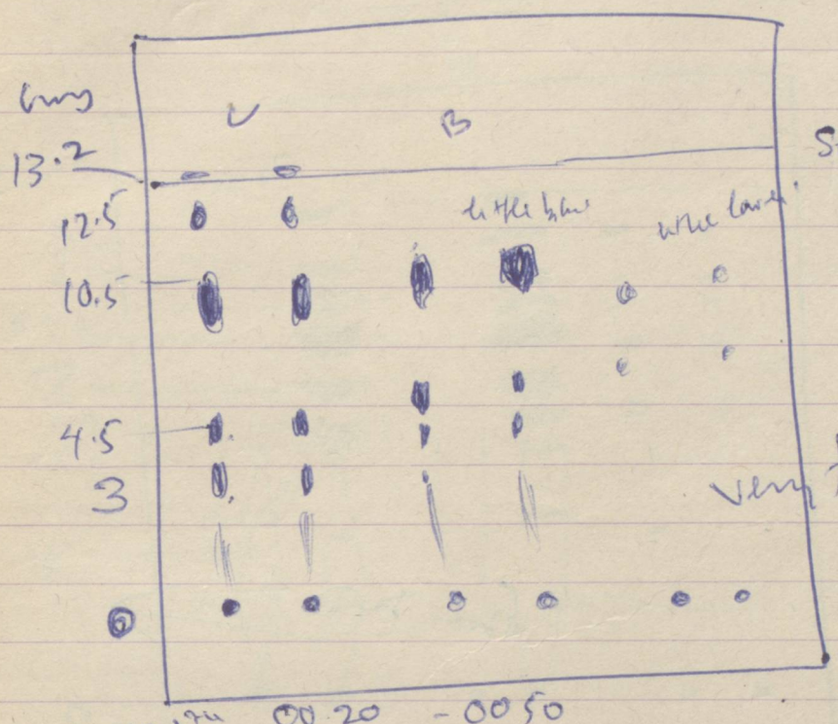
Extract

$CHCl_3$: MeOH 2:1

Solvent

$CHCl_3$: MeOH : H_2O

60 : 25 : 3.5



August 7/8/13th 00.20 - 00.50

12.20 AM - 12.50 AM

00 20 - 0035

12:20 PM - 12.35 AM

$Na^+ K^+$ ATPase

5' incubation

Na^+ Soln						
.3 ml 30% TCA Trimmed	1	11 HC	(K^+ free)	9	11 HC	Trimmed 2.5 ml 5% TCA
	2	S2 B.	(1113)	10	S2 B	
	3	HC		11	HC	
	4	M		12	M	
	5	A		13	A	
	6	B		14	B	
	7	C		15	C	
	8	F	Std (ul)	16	F	
				17	Blank	
				18	Std .01	
				19	.02	

20	11 HC	
21	S2 B	
22	HC	
23	M	
24	A	
25	B	
26	C	
27	F	
Std		
28	Blank	
29	.01	
30	.02	

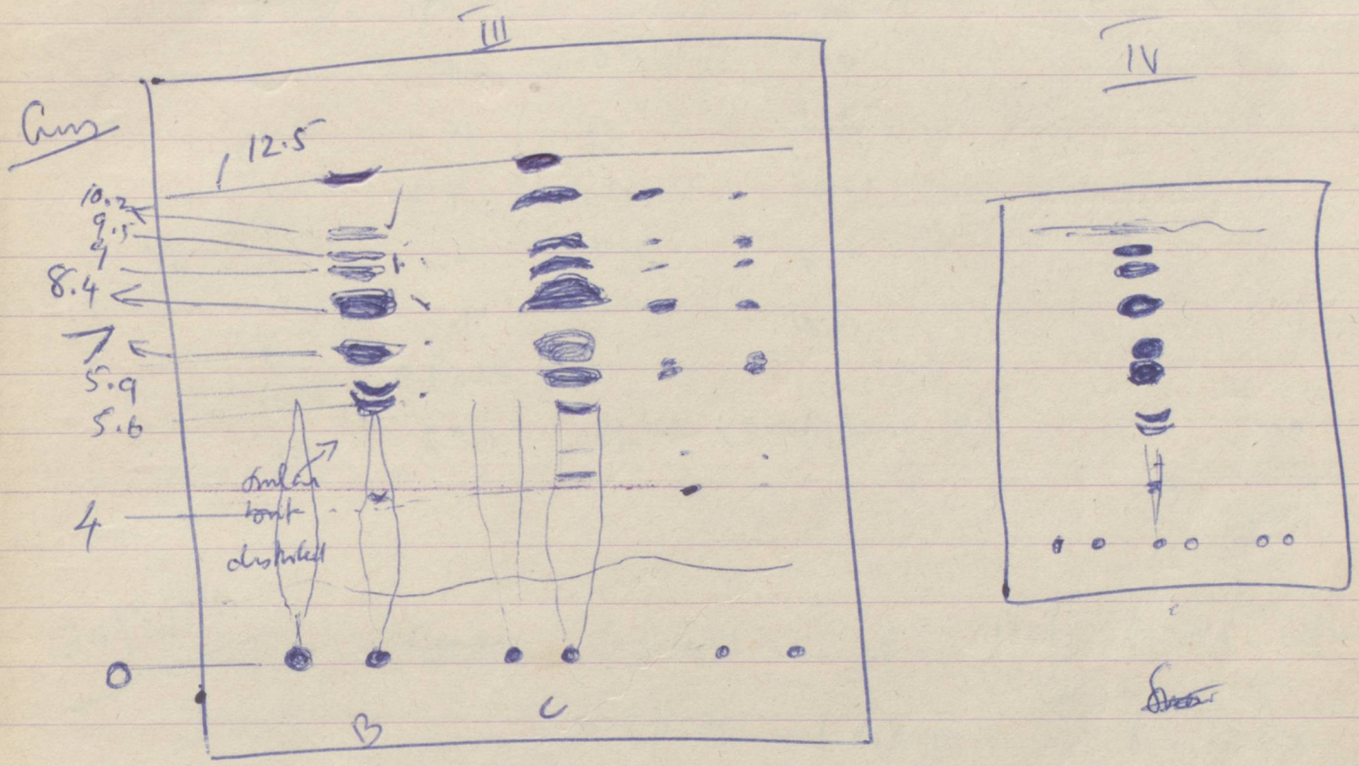
Ina DrB →

Nyut

19/3/74 ~~[illegible]~~ Nyut 1:00

20/3/74 0 - 30

TLC Maïs III & IV



17 Blanc
18 .01 q .1MPi
19 .02 q .1MPi

28 Blanc
29 .01 q .1MPi
30 .02 q .1MPi

Scratch

20/3/74

1 89
2 88
3 101
4 [illegible]
5 64
6 86
7 81
8 83
9 93

10 85
11 102
12 86
13 90
14 87
15 [illegible]
16 84.5
17 [illegible] 72.5
18 94

19 → 116
20 → 72
21 → 72 - 72.5
22 → 78
23 → 72
24 → 72
25 → 66
26 → 66
27 → 70 64 blanc
28 → 88
29 → 106
30 →

Skill Lyme ANBA.

5 ml A + .5 ml B + 1 ml H2O

Proteins

1	HHC	.02	95
2	52B	.02	83
3 (16)	HC	.02	226
4	M	.04	136
5	A	.04	115
6	B	.04	165
7	C	.04	57
8	F	.04	66
9	Blank	0	
10	BSA	.04 ml (300 µg/ml)	21

Left

130

4 × 50 Tari

1/7

20/3/74

Acetone extract & $CHCl_3$: MeOH : water extract.

Lipids (NMR in $CDCl_3$ taken earlier)

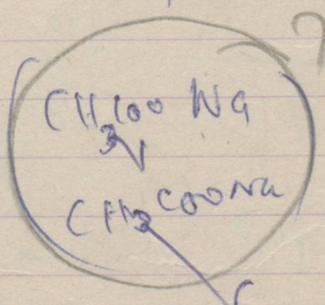
of 6/3/74 Prepared.

TLC

Left 3 spots

Right 4 spots

"



Sod succinate Mol wt 162

64.8 mg in 2 ml .2 M Soln.

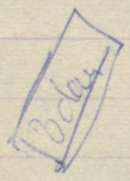
$CH_2-COONa$
$CH_2-COONa$

$C_4H_4O_4Na_2$

48
4
64
46
162

4 NaH_2PO_4

PB



21/3/74

A. Acrolein 35 mg/ml

N : L Arlevenol 20 mg/ml

S : Serotonin 18 mg/ml

16/3/74 preparation

pH 8.5

Blank (cough + A / NS)		Meats + Buffer 8.5 O	Meats + Buffer 8.8 A	Meats + Buffer 8.5 NS		Add	5×1
4		58	55·5	57·5		0	340
I 4	A	55·5	52·0	61·0	(S)	0·01	—
II 4	A	55	52·0	61·5	(S)		480
I 4	A	56	52·5	73·	(S)	+·05	
II 4		55	52	72·			
		(observed pH shift)				4×1	
III 1		62	58	80		Same	
		61	54	91		+·1	340
		62	50	96		+·1	—
							430

Blank examined pH 7.4 340/480

7	55	50	52		
7	54	52 (A)	81	(S)	+·05
7	54	53	> 100		+0.1
7	53·5	53·			
—	53·5	51·5	—		—

21/3/74 Monkey brain – shred a day at 0°C

Preparation: Grey portion from Cortex + half of cerebellum

Washed in Saline & homogenised

0-4°C Homogenised in Tris HCl 7.4 10mM buffer – hand in potter (TRS loose) Spun 4000 rpm 1st RCF B pellet discarded supernatant & fluffy layer – spun at 10,000 rpm 10 mts – pellet diluted by 6 times buffer – layered over 40%. 28%. Linear gradient and spun 24000 rpm (64000 g) in Beckman-L (Gonville Lab) ultracentrifuge collected as membrane.

Dialysed 24 hr (twice change) against 10mM Tris HCl buffer.

Care not to shake at any stage

SINGER.
SR. Insulin assay SDIT 600 hm/M

22/3/24

	H.C (21/3/24)	Casuca Stuff	Mean	HC (16/3/24)	M	A	B
0	40.5	42	43	41	42	42.5	42
15	43.00	42	43.5	42	42.5	43	42.5
30	44.5	42.5	44	42.5	43	43	42.5
45	45	43.00	44.5	43	43.5	43.5	43
60	46	43.00	45	44	44		

OD unit × 1,000 = cell/ml
5 ml × Pathology

MD 11 droplets 357
repeat

		HC (21/3/24) 340	Mean	Stuff Casuca
80	92.5	57	58	55
87	95	60	59	57
94	99	62	59.5	59
99	> 100	63	60.5	61
		64	61	62.5

Transmittance

Transmittance. Calculate 2 mL each standard
for marking (Can not use this Expt) . . . . .
upto Wale as Blank - always.

.16 : 1 µM

.01 x / .16 x .04 x 1/

AMP 11.07 mg in 8 tubes

ATP 3.3 mg in 10 tubes

.0125

Mistake 20% Sod Metabisulfite

(10% Sod Metabisulfite)

15.1. .7.5.1.

23/3/74

Protein estimation — Coleman Spectrophotometer 660 mμ

		Absorbn	Trans.
.05 HC(21) →	1	0.06	87.
.05 Layered ctxyff →	2	0.135	73
.05 Memb(21) →	3	0.18	66.
0 Blank →	4	0	100%.
BSA 0.1 ml →	5	0.285	52.5
BSA 300 μg/ml 0.2 ml →	6	0.47	34.0
0.3 ml →	7	0.335	46.0

Obsab	Trans
0.285	52.5%
0.47	34.0%
0.335	46.0%
0	100%
0.06	87%
0.135	73%
0.18	66%

12-15 - 13.15 1hr (60 mins)

Na⁺ K⁺ ATPase .04ml Prepn 5' 12-15 - 12.45 30 mins nucleohdase 40C

	N	K			
HC(21)	1	.03/93	4	.04/91.5	
L.S(21)	2	.05/89	5	.047/90	
Memb(21)	3	.05/89	6	.06/87	
Blank	7	~~0/100~~		96/.020	
.01 Pi (1M)	8				
.02 ("	9	95/.025			
.03 ("	10	94/.028			

23:35 - 0.35 60'

HC	11	89.5/.05	.04ml
L.S	12	85/.07	Organelle added.
Memb	13	91.5/.04	
Blank	14	100/0	
.01	15	93/.033	
.02	18	94/.028	
.03	17	92.5/.035	

(92.5/.035)

ATPase

.4 + .4 + .16 + .04 + .3 (Tris 24%) + 8 ml + .5 ml + .2 ml. nucleohdase Ps.

1.2 + .04 + 2.5 + 6 ml + 1.5 ml + .2 ml Ps

23:35 - 0.05 30'

(colour didn't develop well) Repeat expt; NaCl same

23/3 / 24/3/74 colour

	Tr/Abs	
1	84/.0775	0.076
2	70.5/.152	0.153
3	82/~~.086~~	0.086
4	82/~~.086~~	0.086
5	65/0.19	0.187
6	68.5/0.165	0.166

540 nm readings

LS: 3 HC M: 9 HC

.57 pgs

Blank 0

1μM .160

2μM .325

7	Blank	100%/0	
8	0.1 (10μM)	12.5%/0.9	100 mucin
9	0.2 (20μM)	10.1/1.0	
10	0.3 (30μM)		

Good readings

11		88.5/0.055	0.055
12		85/0.07	0.071
13		87/0.06	0.061
14	Blank	100%/0	
15	0.1 (10μM)	12/0.925	
16	0.2 (20μM)		
17	0.3 (30μM)		

std: 0; 1M 0.215; 2μM 0.414; 3μ 0.520; 4μ 0.790

(repeat remainder)

Run another standard same time at 540 mμ / same Coma

Fiske-Subbarow Phosphate (Inorganic)
J. Biol chem 1925 66 375

Readings taken after 5 mts & 10 mts
If readings agree O.K.
If readings alter - a third
time after 5 mts. In case change occurs
after 10 mts repeat with lower concentration

10 N. Sulfuric acid (Not 10M)

450 cc of Conc. H_2SO_4 added to 1300 cc
of water.

Molybdate II.

2.5% Ammm molybdate in 3 N
sulfuric acid prepared as above.

(Dissolve 25 gm Molybdate 2.5 gm
in 300 cc of 10N (150 cc of 10M) acid
& dilute to 100 ml with water)

Standard Phosphate Dissolve 0.358 g
of pure anhydrous potassium phosphate in
water (5 cc = 0.4 mg P_i)

15% Sod bisulfite (7.5% Sod meta bisulphite
solution and is free of turbidity if
not clear filter after allowing it to ~~stand down~~
stand for 2 or 3 days

20% Sod sulfate ($CuSO_4 \cdot 7H_2O$)

Arsenate suspended in water

Tetraborate

Dissolve 0.5 gms of dry powder AMSA in 195cc of 15% sod bicarbonate Add 5cc of 20% sod sulphite & bicarbonate Add enough min 5cc of bicarbonate. If not ~~clear~~ ~~keep in~~ keep in expand to 200cc & can be kept for 2 weeks.

4 volume of 10% TCA
1 volume sample

Take 5cc of bicarbonate into the tube Graduated extra 10cc and 10cc volumetric flask add 1cc of 2.5% conc HCl finally add 0.4cc of AMSA reagent Make to 10cc.

24/3/74 Colour ready - ORD-

25/3/74 ———

26/3/74 Freeze dry small flask: 21/3/74 overnation.
large flask 16/3/74.

Marker brain kept in deep freeze on 21/3/74 — all day Perkin — homogenised in Tris — 4000 rpm 1 hr — supernatant thrown away — 8000 rpm 15 min pellet — kept at 0° c for 1 day.

27/3/74. Tris-Hcl buffer 2.4 M.

26/3/74 Buffy coat separated over 1 ml 1 ml PTU
of 90% Ref. Diazo - RC 2B 10,000 rpm
30 min & supernatant collected.
Dialysis on 26/3/74 9:00 AM. 26/...
— 36 hrs.

Method in Enzymology VIII

Glycine - Hcl buffer

A : 0.2 M soln glycine
(15.01 gm / litre)

B : 0.2 M Hcl (~~50 ml~~

50 ml A + x ml B diluted to 100 ml

x	pH	x	pH
5.0	3.6 ✓	16.8	2.8
6.4	3.4	24.2	2.6
8.2	3.2	32.4	2.4 ✓ w enzyme.
11.4	3.0	44.0	2.2

500

Tris - maleate

A 0.2 M Soln Tris acid maleate
(24.29 g Tris 23.2 gm maleic acid
w 19.6 gm maleic anhydride
in 1 litre

B. 0.2 M NaOH
50 ml of A + x ml B. diluted
to 200 ml.

1100

x	pH
7.0	5.2 ✓ w enzyme.
10.8	5.4
15.5	5.6
20.5	5.8
26.0	6.0 ✓

28/3/74 Rat blood in Alsever's Soln. – Centrifuged cells diluted to make up with H_2O.

pellet – added Tris 1/2 hr Centrifuged at 4°C – pellet homogenised – 1/2 hr at 10,000 rpm in RC2B – repeated twice more. pellet – this taken up in resuspending

" Rat brain – homogenised in 0.25M Sucrose (8.5%) diluted to make up with Tris HCl. pH 7.4 hrs 4000 rpm 1 hr – supernatant spun at 19,000 rpm 10 min – pellet. (Blood ⟵ Chromium)

HCR/28 → LSR/28

Diluted by 5 ml buffer. Layered over 10 ml 10 ml. 40/28%. Sucrose spun at 64,000 g 3 hrs. Interphase called M

29.3.74 28 MR dialysed 24 hrs against 2 DW.

1 HR

30 min

	Protein	Stds	Na	$Na^+ + K^+$ ATPase. K	Incubation
1	HCM26	.04	1	8	HCM 21
2	LSM26	.04	2	9	22
3	MM 26	.04	3	10	23
4	HCR28	.04	4	11	24
5	LSR28	.04	5	12	25
6	MR 28	.04	6	13	26
7	CMyR 28	.04	7	14	CMyR 27
8	R Lamb	.04	Standards (KMA)		Studd 28
9	Standard	0.1 ml	Blank	16	
10	Unknown 2	0.2 ml	1 µM 1.01	16	29 1 MM P_i
11	300 µg/ml 3	0.3 ml	2 µM 2.02	17	30 2 µM P_i
12	4	0.4 ml	3 µM 3.03	18	3 30
13	5	0.5 ml	4 µM 4.04	19	
			5 µM 5.05	20	
			6 µM Pi		

1 M .01 10 µM

(1 ml .04 total assay 1 µM 84 1 µM Mg + 13.8 mM Tris in 1 ml)

NaCl

58.44 gm — 1 Ltr — 1M

14.61 gm — " — .25M

2.92 gm — 200ml — "

KCl

74.55 — 1 Ltr — 1M

7.455 — 100ml — 1M

.932 mg — 100 ml — .125

93.2 mg — 100 ml — .0125 M

200 mg/ml

28/3/74 Glycine (Aminoacetic acid) $NH_2CH_2COOH = 75.07$

30.03 gms. in 200ml 0.2M.

① 100ml 2 + 1 ml 0.2M HCl adjust PH 3.5

② " + 1 ml Conc HCl adjust PH = 2.4

30/3/74 one

① Na Succinate 0.2 M. (PH 7.2 2mg/ml) × SRK. mixture.

② NaDH 1 mg in 5 ml. (·4ml / Sample)

③ BSA / egg albumin [30 mg/100 ml] as standard.

DTPase

A. Nacl 0.25M $MgSO_4$ 0.00138M.

B Nacl 0.25M KCl 0.0125M $MgSO_4$ 0.00138M

C. N_2ATP 3.5 mg in 5ml.

D DMP 13.3 mg / 1.2 ml. ½ 12 hrs (nucleic acid).

SDH. 600 mμ.

Cocktail 2.9 ml.

- 1 ml PO_4 buffer PH 7.6 · 1M
- $CaCl_2$ ·8 mM 0.1 ml
- KCN (2mg/10ml) 0.1 ml (mercaptoethanol in KCl.)
- Succinate 0.5M 0.1 ml.
- DCI (1.5mg/20ml) 0.2 ml
- water 1.4 ml.

+ .05 ml PMS. · 600 mM

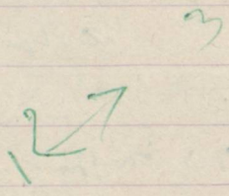
.05 ml Mentp. 15" reading.

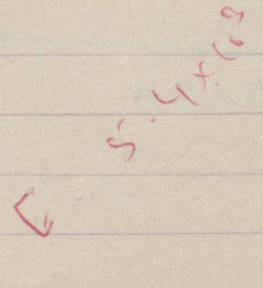
NaDH .4 ml of .02M Tris 7.6 PH

.4 ml NaDH 2mg/10ml in

.2 ml Pot Ferricyanide .003M

.05 ml Mentp.


3
2
1



Blank wali 100% 600 mμ

SDH		0	15	30	45	60	Three
HCM26	1	55	56	57	58	59	
LS26	2	48.5	50	52	54	56.00	
MM26	3	done 50.5 mean 51	51	51	51	51	
HCR28	4	50	52	53.5/54	55	56	
LSR28	5	53.5	54	54.5	55	55.5	
MR28	6	54.5	54.5	54.25/54.5	54.25/54.5	54.25/54.5	
CMYR28	7		not mark				

NADH Diaphorase

Blank wali 340 mμ

	0	15	30	45	60	Three
HCM26 1	42	44	45.5	47	48	
LS26 2	29.5	31	32	33	34	
MM26 3	31	32.25	33.25	33.5	34	
HCR28 4	36.5	38	39.5	40.5	41.5	
LSR28 5	43	44	45	46	47	
MR28 6	45.5	46	46.25	46.25/46.5	46.5	
CMYR28 7	39.5/40	40.5	40.5/41	41	41	

~~.005~~
~~.03 x~~
~~5.4 x 10~~

$\frac{10^3}{90}$ $\frac{5}{90}$ 50 mll.

mmol DCPI

1/mMol Dichlorophenol-Indophenol reduced/ml

See pg 45 for Sample identification

600µm Coleman

Next lot ATP 51

Protein	Ready Transmittance %
1	39
2	18.5
3	36.5
4	40.5
5	47.5
6	79
7	60
8	100
9	69.5
10	55.5
11	41
12	31
13	26

Scratch

Rel prep

+ Enzyme buffer

Complete

1	76		11	57	
2	58		12	60	
3	86		13	90	
4	59		14	65.5	
5	58.5		15	100	
6	89.5		16	59	
7	64.5		17	45.5	
8	60		18	33	
9	63		19	32	
10	82.5		20	26	

51 nucleotides

1	74.5
2	71.5
3	88
4	73.5
5	76
6	94
7	77
8	100
9	69
10	45

	Tr	OD		Tr	OD
1	76		11	57	
2	58		12	60	
3	86		13	90	
4	59		14	65.5	
5	58.5		15	100	
6	89.5		1M 16	59	
7	64.5		2M 17	45.5	
8	60		3M 18	33	
9	63		4M 19	32	
10	87.5		5M 20	26	

pH

½ ml H_2O
½ ml
~~.05M HCl~~ .1 M HCl +

N: 8.4 mg/ml .05M
S: 19.3 mg/ml .05M
Ca: 22 mg/ml 0.2M
A: 36.3 mg/ml 0.2M

K: KIO_3 1N
Na: NaCl 1N

In glucose:

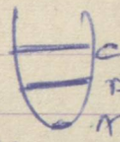
1: 2.5 pH Ethyl-ital buffer + Mans 10% v/v + TNS 10% v/v
2: 3.5 pH Ethyl ital buffer + Mans 10% v/v + TNS 10% v/v
3: 7.4 pH Tris-HCl buffer + Mans 10% v/v + TNS 10% v/v

1B
2B
3B
} Blanks

TNS = TNS in H_2O 3mg/10ml
Mans: BDH B4R

(3) DORD Mars.

3/4/74 4. Rat brains (male-wistar) = 6 gms. washed with Tris. Hcl 7.4 buffer twice. minced in 5.5% Sucrose in Tris 7.4 buffer = ~~50 vol~~. Homogenised in 50 ml 5.5% Sucrose buffer by Dounce-hand homogeniser 100 μ clearance with twenty updown strokes. Whilst turning - Homogenate HC 34 R - Centrifuged at 4000 rpm 1 min - Supt + whitish fluffy layer on top of pellet collected and Centrifuged at 10,000 rpm 10 min pellet resuspended in 4 times buffer. Layered over 40/28%. Sucrose gradient above 6 ml



⟹ discarded 24 hrs.

(No buffer action?)

4/4/74. DH

Buffer. 8.5 g GlyHcl 10 mM 2.5 GlyHcl.

Buffer.	Buffer + Reagents.		Buffer	Buffer + Reagents
7.4 in adjusted to 7.4 ① drops 2.9 in place of 3.5		.01 .04 N 0.2	ine ohne	drops 1.9 in place of 2.5
Buffering Capacity applied directly by dilution		.01 .04 S 0.2		
		.01 .04 A 0.2		
	Repeated by adjustment with buffer	.01 .04 G. 0.2		

4/4/751

Fluorescence.

Excitation 340 mμ
Emission. Blank 460-470
Membs. 430.
① = Ach.
② = Serotonin
③ = Nad IM
Rose.

I

	① Blank.	② Membs	③ Membs + ①	④ Membs + ②	Colour		
Spect. 480-470 = (4) 430 = 2		62.5	58	59.	0	③	5 x 1 peak 430
2	+① a	62.5	66	42 (over 100) (3 x 1/10)	+.01		5 x 1 peak 430 (Max mm reached in 6 mins)
	b	64	69.				
2.5	+② a	66	84	42 (3 x 1/10)	+.01		5 x 1 peak 430
2.5	+③	5 x 1 62.5	(over 100) ~~42 (3 x 1/10)~~ 4 x 1 : 57	40 (3 x 1/10)	+.03 ① +.01 ②		(4 x 1 dark 5 x 1)
3	5 x 1 +①	5 x 1 66/67	4 x 1 : 65	—	+.05 ①		
5 x 1 2.5	5 x 1 +③	76	+③ 4 x 1 : 62	37.5 (3 x 1/10)	+.03 ③		
5 x 1 3	5 x 1 +①	80	+① 4 x 1 : 65		+.03 ①		

I

	①	②	③ +①	④ +②	Colour		
					①	G.	5 x 1 mixed.
					②	N	
					③	K	
2.5		66	64	66	0		
2.5	+①	63	61.5	over 100 4 x 1/10 : 71	+.01		
2.5	+②	63	66	over 100 4 x 1/10 : 69	+.01 ② +.05 ①		
2.5	+①	65	69	—	+.1 ①		
3		65	67.5	—	+.1 ①		
3		64	69	4 x 1/10 : 70	+.04 ③		

Buffer (PH-4) adjusted to PH 4.

(1) 7.4 PH Tris ready 8.7. ~~+ 0.1 N~~

(2) 2.5 PH Glycine HCl 7.7. (+ .01 N PH : 1.8)
+ Veronal 7.4

+ .01 Serotonin PH 1.5 ( )

(1) NaCl ATP Para Veronal acetate

(2) .05 M Glycine buffer (finally) contain .1 ml of .1 M $MgCl_2$
+ ~~0.3 mg~~ 1 mg AMP Final Volume 1.24 ml. Include .04 ml Enzyme.
Terminate by 2.5 ml 5% TCA

Contains ~~1.5~~ mg AMP
25 units 8.3 mg ATP

5/4/74. 600 mμ - Coleman.

Protein		Lowry	
HCR 34 .04	1	13.5	0.878 →
LSR 34 .04	2	45.5	0.345
AR 34 .04	3	46	0.337
» BR 34 .04	4	63	0.201 →
» CR 34 .04	5	51	0.291
Blank	6	100	0
OVal .2	7	75	0.128
.4	8	55	0.260
.6	9	51	0.291 ⊗
.8	10	33	0.489

420 mμ in 20 min

540 mM.

Wzt kt ATPase 3:05 - 4:05

		Na	K		
HCR 34	I	1 0.227	11 0.310	59.5	49
	II	20.229	12 0.326	59	47.5
LSR 34	I	3 0.260	13 0.300	55	50.5
	II	4 0.260	14 0.296	55	51
AR 34	I	5 .116	15 .187	76.5	65
	II	6 .119	16 .168	76	68
BR 34	I	7 0.046	17 0.076	90	84
	II	8 .056	18 .084	88	83
CR 34	I	9 0.051	19 .131 ×	89	74
	II	10 .055	20 .192	88.5	81
Blank		21 = 100%		100	0
1 μM	.01	22		69.5	0.160
2 μM	.02	23		47.5	0.325
3 μM	.03	24		28.0	0.552 ⊗
4 μM	.04	25		13.0	0.886

5' incubation 305 - 335

HCR 34	I	26
	II	27
LSR 34	I	28
	II	29
AR 34	I	30
	II	31
BR 34	I	32
	II	33
CR 34	I	34
	II	35
Blank		36
1 μM	.01	37
2 μM	.02	38
3 μM	.03	39
4 μM	.04	40

Mistake for next page.

with this std. for pg 41

5' nucleotidase 540 mμ

26	HCR34	75	0.125
27	LSR34 + 0.01 Pi	44	0.357
28	AR 34 + 2μM	42	0.377
29	BR34 + 3μM	30	0.523
30	CR34 + 4μM.	21	0.678
31	HCR34	72	0.143
32	LSR 34	99	0.051
33	AR 34	94	0.027
34	BR 34	90	0.046
35	CR 34	87	0.061
36	0μM	100	0
37	1μM	61	0.215
38	2μM	39	0.414
39	3μM	24	0.560
40	4μM.	16.5	0.790

1 1/3

.005 M ATP
.05 M Gly
.01 M $MgCl_2$

.5 ml assay, 5 mM
Add .04 ml Mercl

369 mg / ml . 1 M.
369 / 10 ml = .1 M
36.9 / 10 ml = .01 M
18.45 / 5 ml = .01 M
9.22 / 5 ml = 0.005 M.

10 tubes (9 ml)

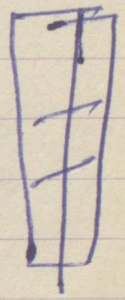
ATP 4 mg / 12 tubes

4/4/74 9H R preparation. Hemolysis in Enzyme (E.S.I.) in Tris HCl
10mM buffer. 40250 1ml 15,000 10' - fraction -
40/28 - 640000 30mS. Dialysis (MD) 24 hrs. and Lab.

9/4/74 Two dimensional Chromatography

5 mg protein 5 ml 6N HCl - 24 hrs 120°C
in sealed tube

Neutralised by NaOH.
Spotted on LH Corner.
1st run Butanol : Acetic acid : water
4 : 1 : 5.
Turned thro' 90°
Phenol : water 4 : 1 (v/v)



Develop by 0.1% Ninhydrin in Acetone

6/4/74 Upper phase 7 4:1:5 Butanol Acetic Acid water system

* Only upper phase *
Colourless phenol.
Ninhydrin - Carcinogen - do not inhale vapours.

Failure. Spots smeared.

Repeat 9/4

11/4/74

600 nm

ml	Nä-K* Puffer			OD
.02	1	HC Na	78	0.112
.02	2	HC Na	77	
.04	3	M Na	86	0.056
.04	4	M Na	94.5	0.026
.02	5	HC K	71	0.149
.02	6	HC K	67	0.089
.04	7	M K	94.5	0.050
.04	8	M K	100	0
	9	Blank		
	10	0.2	85.5	.070
	11	0.4	77	.117
	12	0.6	61	.215

(.2 µM Pi, .4 µM Pi, .6 MM Pi)

5' Incubation					
.04	13	HC	60.5	63	0.143
.04	14	HC	63	63	0.095
.08	15	M	94.5	69.5	0.039
.08	16	M	>100	72	0
	17	Blank	>100 → 100	100	0.056
	18	.2 µM		86	0.111
	19	.4 µM		76	0.174
	20	.6 µM		67	

Blankreaktion ausstellen — nochmal machen

600 nm

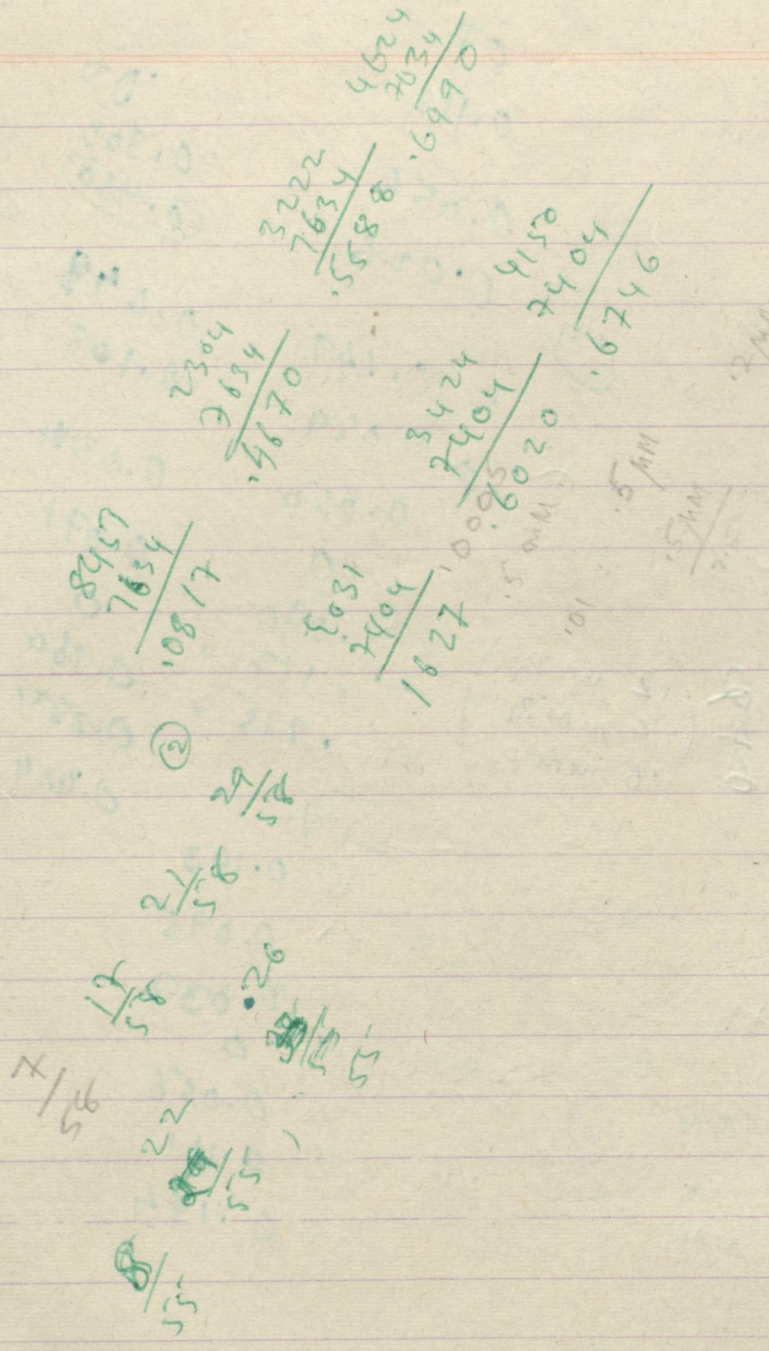
			OD			
	21	.02	0.303			50
	22	.04	0.430	HC		37.5
	23	.02	0.048			90
	24	.04	0.108	M		78
	25	.02	0.034	M HC		93
	26	.04	0.071			85
	27	Blank	0			100
	28	0.2	0.160			69.5
	29	0.4	0.284	(Ovalbumin 300 µg/ml)		52
	30	0.6	0.404			40

(4)

SDH — Enzymaktivierung Assay PMS.

HC	0	15	30	45	60	Reagens Q4
I	48.5	49	50	50.5	51	(.05 ml)
II	49	50	52	53	54	

Muskel MD						
I	49	49	49	49	49	(0.1 ml)
II	48.5	48.5	48.5	48.5	48.5	



2 M' .5 μM 1 5 mM .05 M

12/4/74

N. Orleans N diluted 100 fold .02 .6 2 ml

S. Orleans S diluted 100 fold .02 .6 2 ml.

II B 10 ml 10 mM 7.4 Tris + 1 ml TNS (3.5 mg/ml)/10

I B 10 ml 200 mM 3.5 Tris + 1 ml TNS

II 7.5 ml II B + 0.3 ml M. (N Orleans inoculated)

I 7.5 ml I B + 0.3 ml M (N Orleans inoculated).

2.5 ml 2 cm/cuvette.

① Blank II B	② Native II	③ Native + N II + N	④ Native + S II + S	Δ Ly
6	64	58	64	0
6 + N	64	65	78 (47 6x1)	+.01 7x1
7 + N	68	75	Ova 100 7x1	+.01
			6x1 55	+.01
7 + N	70	79	ova 100 6x1 69	+.01 (S) +.02 (N)
9 + 3N	71	87 + N	6x1 73	+.03
9 + Na	73	86 + Na	6x1 79	+.02 Na (1M)

I B	I	I + N	I + S	Δ Ly
2.	47	49	52.	0 14x1 range
2 + S	47	57	63	+.01
2 + N	47	87	94	+.01
2 + N	50	ova 100 (4x1) 48 (3x1)	ova 100 (4x1) 55 (3x1)	+.01
+ N	50 (4x1)	63 (3x1)	64 (3x1)	+.01

Cnl.

	+ N	+ S
2 +S	50 (4×1)	72 (3×1)	72 (3×1) + 0.05
2 +S	50 (4×1)	79 (3×1	83 (3×1) + 0.1

(+ Ach 10^{-5} M 2 min)

2. 69 (4×1) 83 (3×1) 90 (3×1)

(Grey dam onwards fascia) to 60 // A + 0.1 M 2 min 60 again

(Starch free dam) (Starch dam)

16-17/4/74

T.L.C. DEAE cellulose - chromatography

16 gms in 150 ml water = max limit, barely flowable.

→ 5 plates. Dry in room temp - cool air 24 hrs

Spotted on one corner.

1st dimension. 2% Acetic acid. ! = sample.

Dry. 24 hrs. in air

II nd dimn Butanol : Acetic acid : water

3 : 2 : 1

Dry in air. 1% ninhydrin Spray. - develop at 120°C

19/4/71 RB94 overnight in Locke .3 ml to 7.5 ml bath

I : 3.5 pH bath II 7.4 pH bath 10 mm.

E+ 340
An 430

TTX ~.5 ms/4 ml

II B	II + Arachine	II + N	II + S	
5	49 (6x1)	48 (6x1)	47.5 (6x1)	← 0
5	50	50	51	← +0.01
5	51	55	61	↙ +0.01
5.5	52	58	64	+0.05
5.5	52.5	58 (del TTX)	65	→ (TTX .01 ml.)
5.5	52.5 ↓ Ach. (+.03)	66 ↓ just like	87 just like	+0.05 (NS)
5.5	53.5	66	87.	
5.5	56.5	66	87.	

I B	I	I N	I S	
	6x1 m			0 ↙
5	45	45	46	+.01 ↙
5	45	56	80	+.02 ←
5 (6x1)	45 (6x1)	103 (overload) ← 50 (2x1) ↳	103 (overload) 60 (5x1)	+ ~~.05~~
5 (6x1)	44 (6x1)	74 (6x1) 32 (5x1)	overload 25 (2x1)	+ .1 ml TTX in all (2 = 4)
5 (6x1)	43 (6x1)	after 3 min 71 (6x1)	25 (2x1)	

+ 0.1 ml TTX
in all

5 (6×1)	43 (6×1)	~~77~~ (6×1) 67 (6×1)	13 (2×1)	
5 (6×1	43 (6×1)	over 100 56 (2×1)	over 100 75 (2×1)	+ 0.05 ANS " " 2 3 4 " "
5	48 (6×1)	60 (2×1)	79 (2×1)	+ 0.1 A in 2 Rest-Recept-
5.	48	~~60~~ (2×1) 59 ↗	67 (2×1)	+ TTX 1 ml in 3 & 4

2

(TTX in 10mM Tris HCl) 4 hrs

22/4/74 4 Rats. usual procedure A B C in succession

B - part dialysed **RB224**

Fractions in succession undialysed.

I B 10 ml buffer + 1 ml TNS. | -> 2.5 ml 1 hr | PH 3.5
I 8.5 ml of I B + 0.3 ml mems B. | 10 ml

II B / II | Same as above. buffer PH 7.4 · 10 ml

III B / III | Same as I B I | treated with 0.1 TTX dialysed buffer.

IV B / IV | Same as I B I | treated with 0.5 ml TTX.

V B / V | Same as II B II | treated with 0.1 ml TTX

Dialysed B. for 24 hrs. setting?!! — for IR.
and etc.

Cubes I. native
II. Ach. (uncadmium)
III - N. (drop)
IV - S. (drop)
V G. drop)

→ Polymerise IS polymerise

SDH

	0	15	30	45	60 seconds
B	46	46	46	46	46
A.	48.5	49.5	51	52	53

Excitation 340? Emission 430. Iberian records

360?–430 Mens. Dean 340–480

I B	I	I +A	I +N	Qty
2 (4×1)	36 (4×1)	40 (4×1)	38.5 (4×1)	0 (A doubled)
2 +N	38.	47.5	55	+0.01
2 +N	38.5	55	Over 100 46 (3×1/10)	+0.03
2 +N	40	62.5	60 (3×1/10)	+0.06
Just repl	+Na	+S	+K	
2.	42	52.5 (5×1/10)	61 (3×1/10)	.01 .03
2.	47	63.5 (4×1/10)	6.5 (3×1/10)	.06
2	51	53 (3×1/10)	60.5 (3×1/10)	

III B	III	III +A	III +N	
	Blank 340–480	Mens 360–430		
2 (4×1)	44	38	37	0
2 +N	44	48.5	84 (4×1)	.01 .03
2 +N	44	56.5 (4×1)	56 (3×1/10)	
Just-	+Na	+S	+K	
2	45	54. (5×1/10)	56 (3×1/10)	.01
2	51	85 (4×1/10)	43 (3×1/10)	.03
2	60 (4×1)	59 (3×1/10)	39 (3×1/10)	.06
2	Over 100 60 (3×1)	58 (3×1/10) Norm added	30 (3×1/10)	+0.1

IV B	$\overline{IV}$	$\overline{IV}_{+A}$	$\overline{IV}_{+N}$	
2	51	50	49	0
2^{+N}	50.5⁻	55 (4×1)	60.5⁻ (4×1)	+0.01
2	50	59	~~76~~ (4×1/10)	+0.03
			15 (4×1/10)	** Shaley
			45 (6×1/10)	turbidite
2	50 (4×1)	60 (4×1)	60 (3×1/10)	+0.06
kept	+ Na	+ S	+ K	
2	51	45 (2×1)	56 (3×1/10)	.01
2	55	67 (4×1/10)	56 (3×1/10)	.03
2	75⁻ ✓	50 (3×1/10)	52 (3×1/10)	.06 (~~Ca, Mg~~)
2	79	52 (3×1/10)	45 (3×1/10)	0.1 (Na, Kaolinite due to turbidite)
		Turbidite	Turbidite	
2	Overlow 65 (3×1)	52 (3×1/10)	36 (3×1/10)	+.1 Na ans

7L60c1 0212 1135
7924 7924 7924
.9480 .2268 .3215

Blanks 340-450 | Blanks 360-430

II B	II	II +A	II +N	Ek
9.5 (6x1)	62	62	61.5	0
9.5 +N (6x1)	62	67.5	62.	+0.01
9.5 +N	61.5	72	65	+0.03
9.5	63	76	67.5	+0.06
Antkupf	+Na	+S	+K	
9.5	63	76	66.	+.01
9.5	67	75	68	+0.03
9.5	ovalen 64 (5x1)	75.5 (6x1)	75 (6x1)	+0.1

V B	V	V +G	V +N	Ek
9.5 (6x1)	60	62.5	62.5	0
9.5	60	62.5	64	+0.01
9.5	59.5	62	64.5	+0.03
9.5	60	63	66.5	+0.1
Antkupf	+Na	+K	(Kupfer)	
9.5	60.5 Na	60 K	63. (kupfer stech NOK)	+0.1
9.5	63.5 (6x1)	60.5 (6x1)	62.	+0.03
9.5	56 (5x1)	68 (6x1)	63	+0.1
9.5	63 (5x1)	73 (6x1)	61	+0.1

(9.6 ms.)

ATP 8 mg / 24 tubes + 2 blanks include stds (2 acc.)

AMP: ~~13.88 (13.86 ms)~~ 9.22 / 5 ml. 13.86 / 7.5 ml. | 15 mg / 8 ml.

1.5 ml 7 hrs / inst.

add alg. Mg/Ca to make 0.05 M & 0.01 M.

30 mls

Na⁺ K⁺ ATPase

SHO mM

B not BD

15 mls

Nucleotides

Na⁺

Hc	1	90x	.046				
	2	~~26~~	0.020				
Mems	3	64.5	0.160				
	4	x 73					
N	5	x 91					
	6	87	0.061				
G	7	63	0.198				
	8	64					
A	9	64x					
	10	72x	0.143				

Na⁺ + K⁺

		89x	0.051
Hc	11	91.5	0.040
	12		
Mems	13	63.5	0.200
	14	x 69	
N	15	76.5	
	16	76	0.122
G	17	~~62~~	
	18	63	0.210
A	19		
	20	74x	0.197

Na⁺ 5

K⁺

	21		23				
	22		24				
	2	88	0.056				
Na5	22	84x	0.0				
	23	87.0					
K5	24	90x					
0	25	100	0				
	21	95	0.022				
	91	0.044					
	27						

25 0 mM → 27 0.2 μM →

26 .1 mM →

Hc	1	94	
	2	94	0.027
	3	84x	
Mems	4	83	0.083
	5	83	0.083
N	6	98x	
G	7	81	0.093
	8	94x	0.028
A	9	90x	0.047
	10	82	0.087
	11	95	0.021
S	12	93.5	0.031
	13	100	0
0.1	14	96	0.019
0.2	15	90.5	0.045

5 Comp 1+5+.5 ml

protein	Tn.	OD.
Hc 1 .05	70	0.155
Hc 2 .05	70	0.155
M(B) 3 .05	49	0.315
M(B) 4 .05	48	
Blank 5	100	0
.2 Ovalbumin 6	77	0.115
.4 7	61	0.215
.6 8	51.5	0.290

0.315

120 of 3+5 +5 ml
+0.315
245
+0403
4-03

300
+200 + 3/4

ANS – dissolved in water – boiling
little alcohol added kept in refrigerator for 3 days – needle like crystals – dried with filter paper

3 mg weighed – dissolved in 10 ml – 1 ml of this to 10 ml buffer 4 ml 7.5 ml + 0.5 ml of membrane preparation (about 250 µg protein/ml in final solution)

100 µM ANS Conc.

Student

9(10)/5/74 Rat brains (four). Kept in freezer for a week - homogenised in 8% sucrose in Tris-Hcl 7.4, 10 mM buffer 40/28 multiphase B. Dialysed 16 hrs B.D. A. below 40°. Crude Homogenate Hc

Ex G: 20.6 mg Gaba/ml soln in water (0.2M)

A = 36.3 mg Acholine/ml soln in water (0.2M)

(1.7 mg in 0.2 ml) N = 8.5 mg L-Artrenol/ml in 1 ml of 0.05 M Hcl (0.05M)

(4 mg in 0.2 ml) S = 19.4 mg Serotonin/ml in 1 ml 0.05 M Hcl (0.05M)

($\frac{N}{100}$ dilute N 100 fold.)

($\frac{S}{100}$ dilute S 100 fold.)

Na = 0.2 M Na.

~~Sample~~ Blank PH 7.4 Tris-Hcl 10 mM buffer. 10 ml + 1 ml TNS (0.3 mg/ml)

11/5/74

Sample 1 ml (BD or B) to 8 ml of above.

(roughly 500 µg protein/ml)

0.2 ml Hc to 8 ml Blank

0.1 ml A to 8 ml Blank

Blank: Excitation 340 (to check this) via experiment with ANS)

Emission: 480 (peak)!

Blank always peak at 480.

Samples Exhaustion 340 Emission 430

B - undialysed.

① Blank	② Mems	③ Mems + A	④ Mems + N/100	Qty added (ádd)
1.5 (6×1/10)	59	58	57	0
1.5	59	66	57	0.01
1.5	59	74.5	58	0.02
1.5	59	79.5	59.5	0.03
	+ Na	+ S/100	+ G	
1.5	70.5	80.5	62.5	0.02
			Just kept	
1.5	78.5	80.5	58.0	0.03

B.D. Dialysed 16 hrs 340-430

①	②	③ + A	④ + S/100	Qty
1.0 (6×1/10)	58.5	55.5	57	0
1.0	59.5	59	55	0.01
1.0	59	63	56	0.02
1.0	58.5	80.5	58.5	0.03
	+ Na	kept		
1.0	61	81	58	0.02
1.0	68	80.5	58	0.03

A & HC

0 H.C	② + A	③ A	④ + A	Qty
42	45	33	32	0
41	44.5	33	32	0.01
40.5	44	32.5	32.5	0.025
42	48	33.5	38.5	0.05
+ Na	kept	+ Na	kept	
44.5	47.5	33.5	38.0	0.05

11/5/74 $Na^+ + K^+$ ATPase — Centrifuged ~~at~~ Glow developing

30 mts

1	Na^+ HC	60·5/0·22	560 mμ
2	K^+	55·5/0·255	
3	Na^+ B	72/0·14·	
4	K^+	62·5/0·205	
5	Blank	100/0	

Protein

I	Blank	100/0	
2	0·2 BSA 60 μg	78/0·105	
3.	0·4 BSA 120 μg	61·5/0·21	
4	0·6 BSA 180 μg.	47·5/0·325	
5	0·02 HC	31/0·505	
6	0·02 A·	34·5/0·46	
7	0·05 B	29·5/0·53	
8	0·05 BD·	43/0·37	(less Folin's reagt. Calc B reading)

12/5/74 PH 7 adjusted — Hread 3·8·

PH paper indicator

I	Buffer (Na-Hcl 3·5)	5·8	
II	Buffer + Mems	7·4	
III	" + 0·05 N	2·2	2·5–2·8
IV	" + 0·05 S	2·4	2·5–2·8
V	" + 0·1 A	6·4	6·00
VI	" + 0·1 G	7·5	7·3

13/5/74

I. I B ANS 0.3 mg/ml 1ml to 6ml buffer. PH7.4 10mM Tris Hcl

I 0.5ml B to 7.5ml above.

II II B I B + 0.02 ml TTX to 10ml.

II I + 0.02 ml TTX to 10ml.

III III B I B + 0.1 ml TTX to 10ml

III I + 0.1 ml TTX to 10ml.

IV IV B I B + 0.5 ml TTX to 10ml

IV I + 0.5 ml TTX to 10ml.

Blank

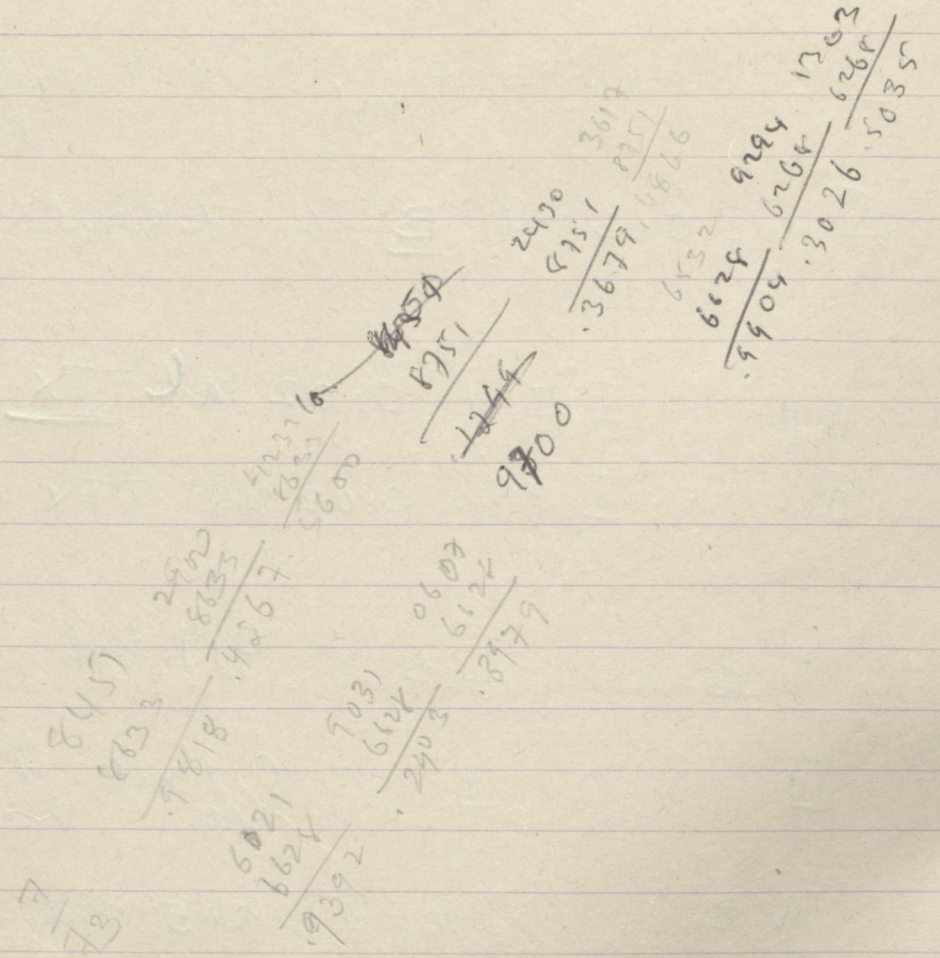
Excitation 370 (maximal emission) 3x10 range

380	over 100		
390	6	510	49
400	2	520	47.5
410	2	530	44
420	4	540	38.5
430	5.5	550	33
440	6.0	560	27.5
450	9.0	570	22.5
460	16.0	580	15
470	24.0	590	9.0
480	34.5	600	5
490	42.5	610	3.0
500	47.5	620	1.5

+Membrane.

Excitation 390 M max emission (4x1 range)

420	36
430	38.5
440	47.5
450	61
460	68
470	72.5
480	70
490	63
500	54
510	45
520	36
530	27.5
540	21



Sample.

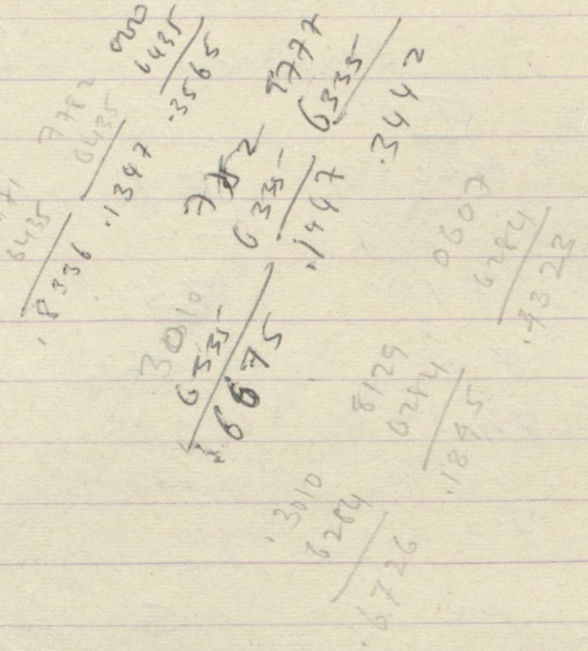
Excitation 390

Emission 470

I B Blank.	I Meth	I + A	I + N/100	Qty
4	71	75.	71	0
4	74	85 (-3)	75·5	0·01
4	74·5	96 (-3·5) (3x1=52)	77	0·02
4	73	100 (-2) (3x1=54)	75	0·03
	+ Na	+ S	G	
4	80	3x1 = 70	76	0·01
4	92·5	over 100 3x 1/10 = 50	79·5	0·02
4	99·5	(turbidity)	79·5	0·03

↙ 3x1 range ↘

II ① B	II ②	II ③	II ④	Qty
		+ A	+ G	
	(4x1=87)			
3	46	46	45·5	0
3	46	50·5	46·5	0·01
3	45	53·5 +1	44·5	0·02
3	46	59·5	46	0·03
	+ Na	Kept.	+ N	
3	50	59·5	54	·01
3	53	58·5	over 100 57 (4x 1/10)	·02
3	57	59		0·03



III B ①	III ②	III ③	III ④	Q 4
	Memb	+A	+G.	
2.5	42	44	43	0
2.5	42	47	43.5	0.01
2.5	43	51	43	0.02
2.5	42.5	54.5	43	0.03
	+Na	kept	kept	
2.5	44.5	54.5	43	.01
2.5	51	56.5	44.5-45	.02
2.5	55	55.5	44.5	.03

IV ① B	IV ②	IV ③ +A	IV ④ +G.	Q 4
3	43	43	43	0
3	41	45	42	0.01
3	41	49	41	0.02
3	41	52.5	41	0.03
	+Na	Just kept		
3	43	52.5	41	.01
3	48	54.5	42	.02
3	53	55	42	.03
		+N	+S	
3	54.5	60	62	.02

14/5/74 ANS labelled 1 ml ANS to 10 ml buffer.

Hc 0.2 ml 9 ml buffer A: 0.2 ml to 9 ml buffer.

Hc 4 x 1 range. 390-470 o/c. checked.

Blank	Hc	Hc + A	Hc + Na	Qty
3.5	34.5	32	33.5	0
3.5	35	33	34	+ 0.01
3.5	34	33.5	34	+ 0.02
3.5	34.5	37	35	+ 0.03
	+ N	+ G	kept	
3.5	OVR 100 3 x 1/10 52	38.5	37.5	+ 0.03

(no stirring & Na calc/ml water not in what is in buffer)

A 4 x 1 range

Blank	A	A + A	A + Na	Qty
3.5	45.5	43	45	0
3.5	44.5	43.5	44.5	0.01
3.5	45	50	46	0.02
	G.	kept	Na	
3.5	45.5	51.5	48	0.03
3.5	45	51	49.5	0.05

16/5/74

Monkey - young one - Grey Cortical regions roughly & grey - homogenised in 60 ml of 0.5% Sucrose in Tris-HCl 7.4 PH 10 mM buffer - Centrifuged 3000 rpm 1 mt - Pellet discarded - Supt + little fluffy stuff Centrifuged 10,000 rpm 10 mts - Pellets from 6 cups suspended in 30 ml of Sucrose-less buffer. - LSM165". Layered over 40/28 Sucrose discontinuous gradient - Spun 24,000 rpm (64,000 g) for 45 mts. Myelin phase collected as B. - MB165. above 28% MC165"

~~MB/4/16~~

16/5/74 Rat brain membrane of 11/5/74 freeze dried Extracted lipid $CHCl_3$: MeOH : Water 8 : 4 : 1

Lipid dissolved in $CHCl_3$ phase - calculated 2 mg Sonicated in 4 ml water 1.5 ml to 8 ml buffer with TNS (1 ml 0.3 mg/ml TNS to 10 ml buffer as blank)

Blank. Maximum emission for excitation at 360

360 excite $12 \times \frac{1}{10}$ (multiplier)

380	31	
90	31	
400	38	
10	42.5	
20	42	
30	40	
40	42	
50	46.5	
60	50.5	
70	53.5	
80	54	
90	51.5	
500		
10		
20		
30		
40		
50		
60		
70		
80		
90		
600		
10		
20		

Blank (Emission)

500	48
10	43
20	37
30	32
40	26.5
50	22.5
60	18.5
70	14.5
80	10
90	6.5
600	
10	3
20	2.5

~~RBL115~~

Blank (12 x 1/10 range)

Emission 480

Excitation

250	5
60	6
70	9
80	15
90	21
300	26
10	31
20	37.5
30	47.5
40	51.5
50	54
60	56
70	52.5
80	39
90	27
400	18.5
10	15.5
20	10
30	7
40	6.5
50	7

Emission 410

Excitation

250	4.5
60	5
70	7.5
80	16.5
90	26
300	25
10	19.5
20	17
30	23
40	31.5
50	40
60	46
70	33.5
80	18
90	lowered

(Blank archive here)

Sample RBL 115 Sonicated in [illegible] + Sulfo

(4 x 1 range)

370. Maximum emission for Excitation?

Excitation 370		Emission 430	
~~Emission~~		~~Excitation~~	
390	over 100	250	8
400	66	60	6.5
10	68	70	10
20	72	80	14
30	73	90	16.5
40	69	300	20.5
50	62	10	25.5
60	54	20	34
70	47.5	30	42.5
80	37.5	40	54.5
90	30	50	61
500	23	60	70.5
10	16	70	75
20	14.5	80	63.5
30	11	90	52.5
40	9	400	53
50	7	10	over 100

Excitation 370 Emission 430.
(Add dansyl-glycine)

3×1 range.

① Blank	② RBL116	③ RBL115 + A	④ RBL115 + Na	Qty
1.5⁻	45	42	43.5⁻	0
1.5⁻	45.5	43	47	0.01
1.5⁻	47	45⁻	51	0.02
1.5⁻	46.5	46	53	0.03
	+ A	+ S	Repl-	
1.5⁻	50	77 in (1×1)	53	0.03
	Repl-	over 100 (3×1) Repl-	+ Na	
1.5⁻	49.5	77 (1×1)	59	0.05⁻

17/5/74

I | IB 10 ml buffer + 1 ml TNS (·3 mg/ml) + Sucrose to give 3%
Sucrose blank
IB (Sucrose test) 8 ml + 0.5⁻ ml MB165

TNS in PH 7.4

II | I (ord blank) IB + TTX 0.02 ml to 10 ml

III | I (Sucrose blank) IB + TTX 0.1 ml to 10 ml

IV | I / IB + TTX 0.5⁻ ml to 10 ml

ANS pH 7.4

V
VI Sucrose blank Same as above ANS in Place of TNS
VII
VIII Sucrose blank.

IX } Ovalbumin
X } ~~BSA~~ in TNS & ANS
(1.5 ml of 300 µg/ml Ovalbumin)
& 1.5 ml bright + DNS & TNS
(1 ml & 1 ml)

I Blank (I B)

5 x 10 range

360 Excitation 480 Emission Maximum emission

360 Excite. / Emission

390	43
400	52
10	58
20	57
30	54
40	55
50	59
60	65
70	69
80	70.5
90	68.5
500	64
510	58

Emission 480

Excite

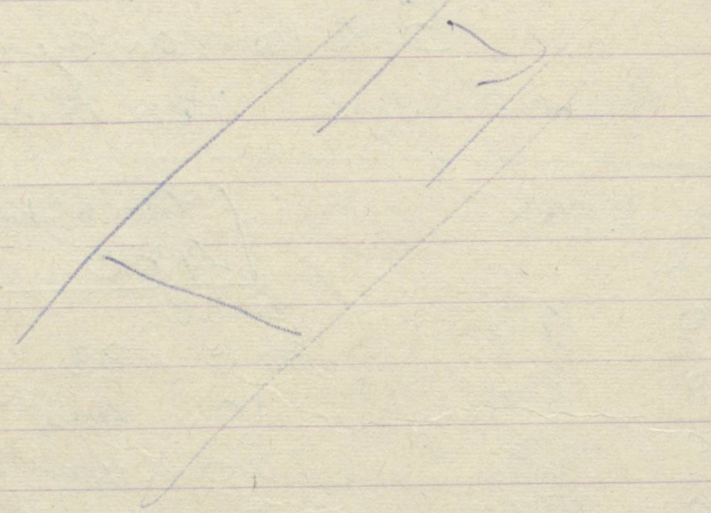
300	33
10	40.5
20	49
30	64
40	69
50	71
60	74
70	69
80	51
90	32
400	19

Emission 410

Excite

260	5.5
70	9.5
80	22
90	41
300	43.5
10	37
20	29
30	37
40	51
50	66
60	78
70	72
80	33

6490
7559
.1931



3.5
59.5

5441
7597
.7844

1.000
1.3747
.2413
.0212
.2625

13.5) 1303
3555
.3744

3324
7554
.5765

4698
7554
.7144

I : Mens + I B 3 x 1 ramp.

Exatahi 360

Anahsr	
380	68
90	38
400	50
10	63
20	73
30	77
40	74
50	66
60	57
70	44
80	38.5
90	30.
500	23.5

Anahsr 430

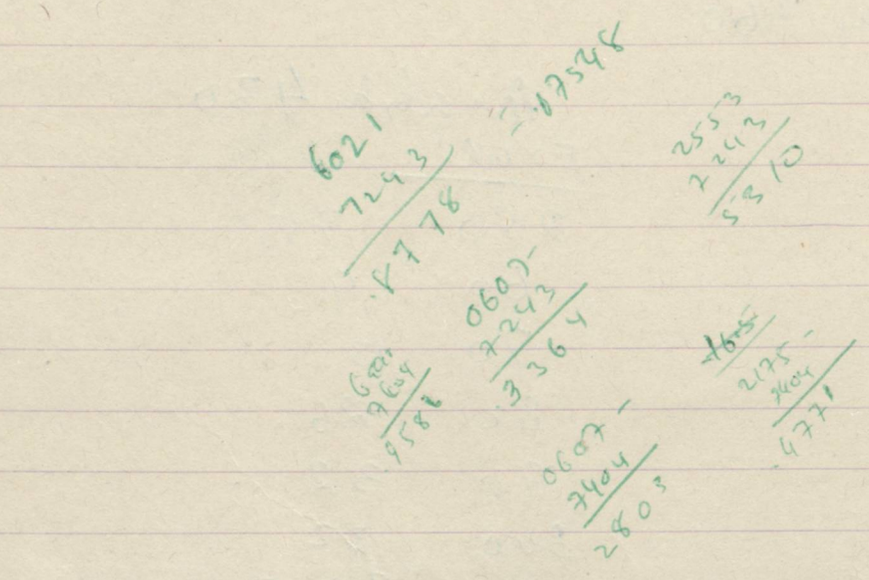
Exali

250	75
60	9
70	13
80	26
90	32
300	32
10	38.5
20	52
30	65
40	66
50	68.5
60	76.5
70	78
80	60.5
90	34.5
400	29.5

---

360 - 430.

I / I B	I	I +K	I +Na	Kl
2.5	75.5	57.5	57	0
2.5	77.5	63 (-2)	64 (-2)	0.01
2.5 +A	76.5	65.5 (-1)	71.5 (-1)	0.02
2.5	75.5	67.5	78.5	0.03
2.5	75.5	68	86.5	0.04
	+G	Icepl-	Icepl-	
2.5	78.5	70.5	91	0.02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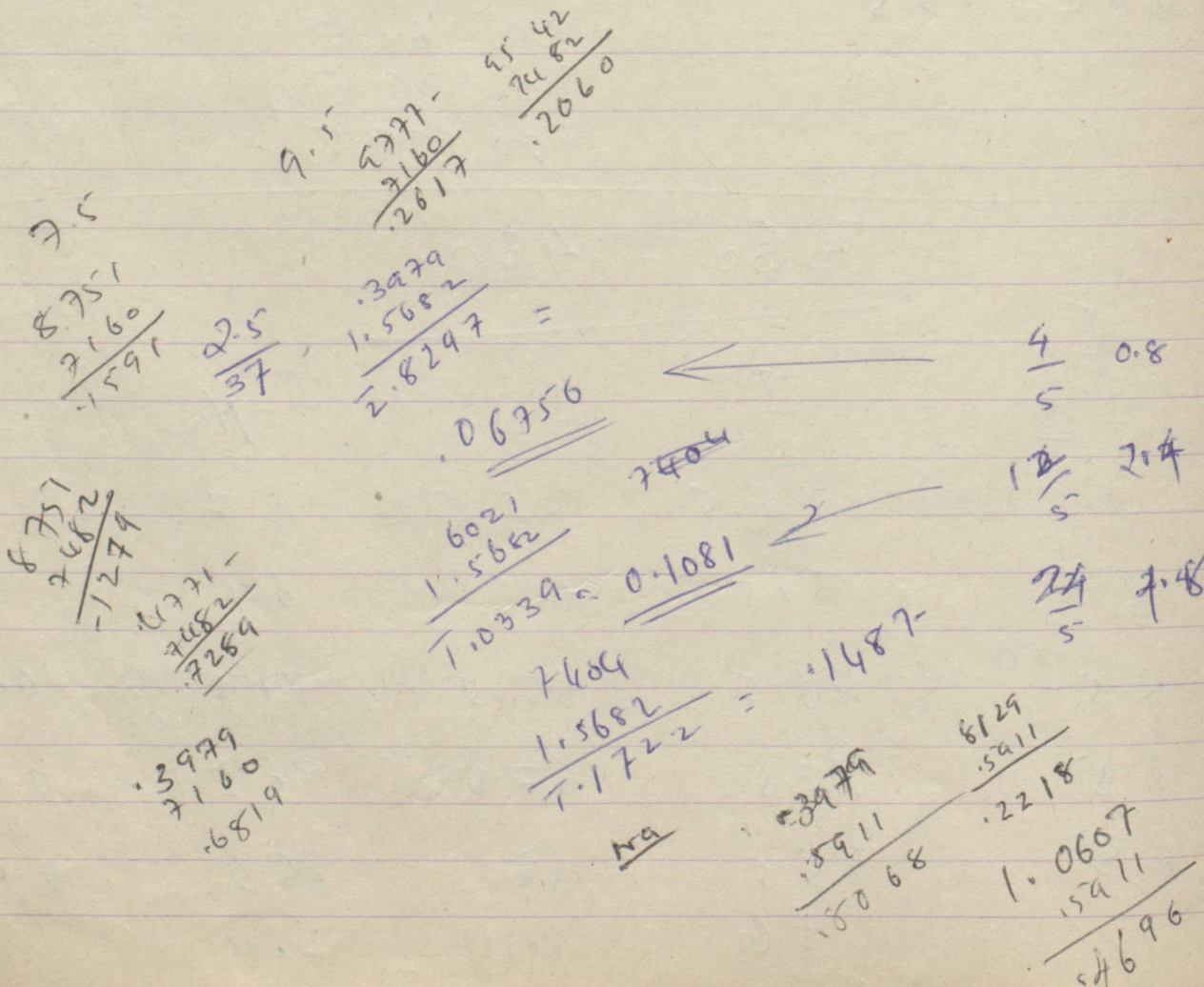
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8.751
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37
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2.8297
=
.06756
4
5
0.8
7404
6021
1.5682
1.0339
0.1081
7.4
7.48
8.751
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.6819
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1.1722
= .1487
NG
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.8129
.5911
.2218
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.5911
.4696

3 x 1 rang · 0.02 TTX in 1 ml

II B	II	II +A	II +Na	Qh
3.	53	52	53	0.0
3.	50.5	53 +2.5	54.5 +2.5	0.01
2.5	49.5	56 +2.5	61 +3.5	0.02
2.5	50	58.5 +3.	68 +3	0.03

3 x 1 rang

III B	III	III +A	III +Na	Qh
2	58	56	55	0.0
2	56	57.5 +2	58	0.01
2	55.5	61 +2.5	64	0.02
2	56	63 +2	71.5	0.03

390 - 470 4 x 1 rang

V B	V	V +A	V +Na	Qh
3.5	39.5	37	39	0.0
3.5	39.5	39.5	41.5	0.01
3.5	39.5	41	45.5	0.02
3.5	29	43 +2.5	50 (+5)	0.03

.0621"

12.5"
410

(2) TNS + BSA 4x10

Analysis 410

Exclusive.

250 1.5"
4.5"
13.5"
38.5"
51
34
310 21
16
18
22
30.5"
41
370 33.5"
11
320 18
400 Over 100

(3) TNS + BSA

Exclusive 360

Analysis

380 6
90 0
400 2
6.5"
7.5"
8.5"
9.5"
13.5"
18
24.5"
21.5"
37
29
34.5"
36.5
32.5
27
22

Analysis 510

Exclusive

260 2
70 4
8
11
300 10.5"
10.5"
14
330 21
29
35
58.5"
40
37.5"
31.5"
21
11
5"
2
1
1

4 x 1 range (390-470)

VIII B	VIII	VIII +A	VIII +Na	Qty
4.0	40	40	40	0.0
4.0	40	41	42.5	0.01
4.0	40	44.5	47	0.02
4.0	39.5	45.5	51	0.03

Ovalbumin TNS ② ANS ③

Blank ANS.

390-470 4 x 1 range

Blank 3.5 ③ - 3.5.

No exhaustion

4 x 10 range.

① Blank. ANS.

Analyte: 510

Analyte	
300	5
10	6.5
20	12
30	24.5
40	36
50	45.5
60	52
70	53.5
80	51
90	42
400	21
410	6.

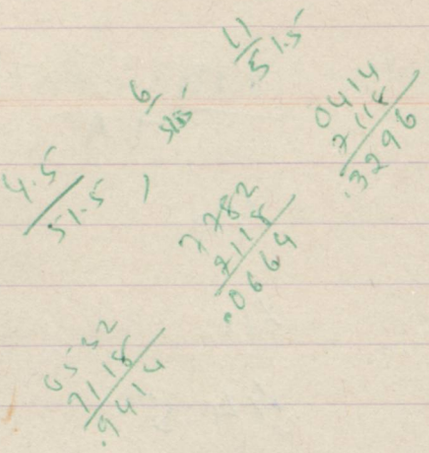
Exclude 370

Analyte	
390	6
400	0
10	2
20	5.5
30	7
40	7.5
50	12
60	19
70	29
80	39
90	48
500	53
10	54
20	51.5
30	46
40	39.5
50	34
60	27

② TNS + Ovalbum

Exclude 360

Analyte: 22	
360	20
90	26.5
400	29.5
410	29
20	26.5
30	→
40	20
50	25.5
60	25
70	25
80	24.5
90	22.5
500	20
510	17.5
20	14.5



TNS 360-430 3x1 range

IV B	IV	IV +A	IV +Wa	Q4
2	59.5	55	51.5	0.0
2	62	58 -2.5	58.5	0.01
2	62	60.5 -2.5	60	0.02
2	65	64 -5.5	68	0.03
	+5	repl.	repl.	
2	101	62	67	0.02

2nd cuvette spectrum (excited mercury)

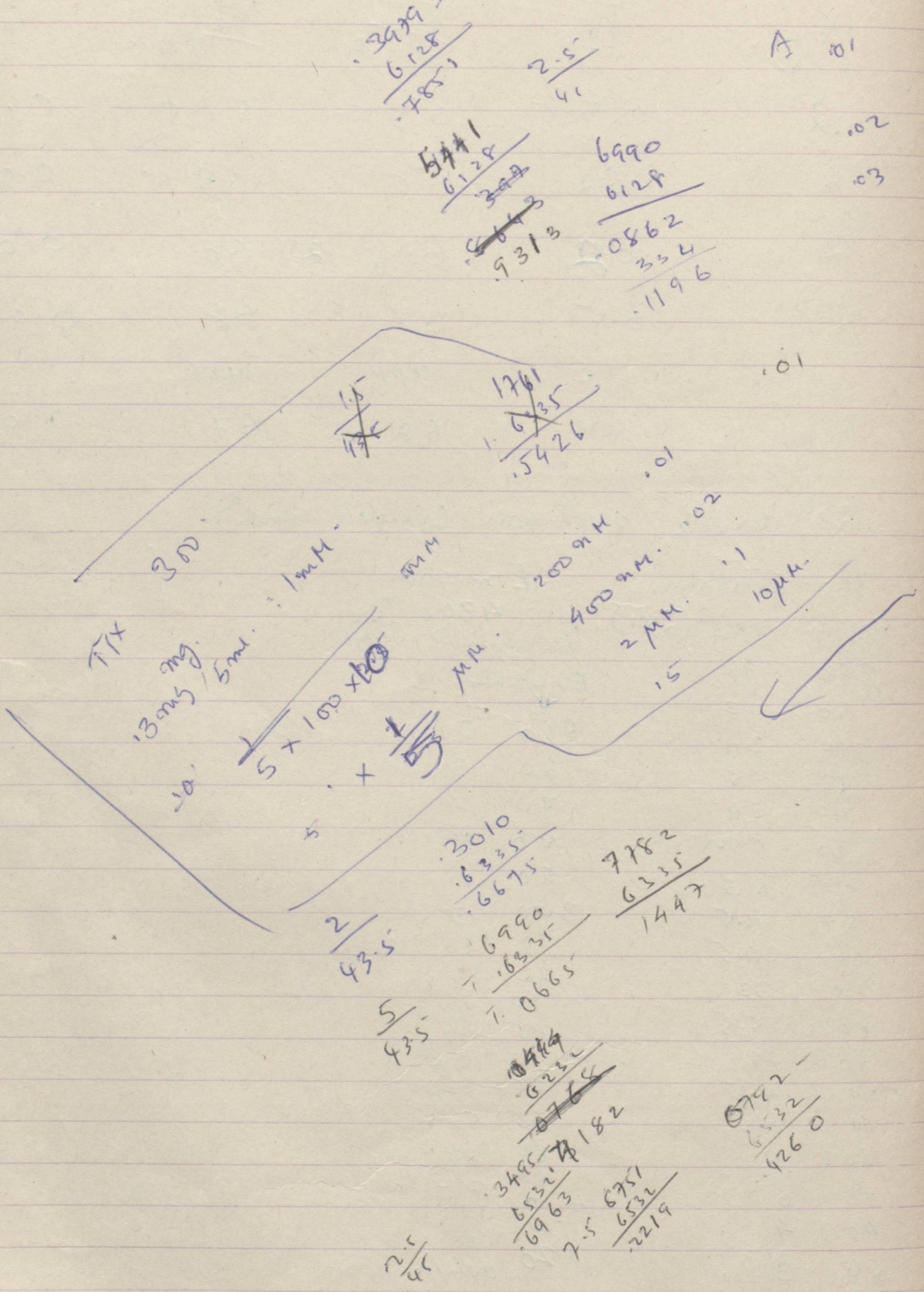
360 Excitation (2x1 range)

Analyzer	Exc. Mean
380	45
90	23
400	34
10	45
20	54
30	55.5
40	53
50	46.5
60	39.5
70	33
80	25.5
90	19
500	14.5
10	10.5
20	8
30	6
40	4
50	3

Emission 430 λ (2x1 range)

(Analyzer) Excitation

250	2
60	3
70	4
80	7
90	10
300	13
10	20
20	34.5
30	48.5
40	47.5
50	49
60	54
70	53.5
80	38
90	22
400	12.5
10	36
20	overflow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41
A .01
.02
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6128
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334
.1196
.01
1.5
43
1761
6335
.5426
.01
.02
.1
.15
300
1mM
mM
200 nM
400 nM
2 μM
10 μM
TIX
.3 cms
mg.
5 ml
10
5 x 100 x 10
5
mM
.3010
6335
.6675
6990
6335
.0665
7782
6335
.1447
2
43.5
5
43.5
.0444
6232
.0766
.3495
6532
.6963
.0182
7.5 6751
6532
.2219
.0792
6532
.4260
2.5
41

VI ANS TTX 0.02 to 10 ml

4 x 1 range 390 - 470

VI B	VI	VI + A	VI + Na	Qty
4.5	42	41	42	0.0
4.5	42	43.5	45	0.01
4.5	41.5	44	47	0.02
4.5	41.5	46	52	0.03

VII ANS TTX 0.1 ml to 10 ml 4 x 1 rang

VII B	VII	VII + A	VII + Na	Qty
3.5	45	43.5	45	0
3.5	44.5	45	47.5	0.01
3.5	44.5	48	52	0.02
3.5	44	49	56	0.03

Lakshman Synaptosomes 0.2 ml (sediment) + 10 ml buffer + 1 ml ANS

G. double. (All usual dosings)

(1) Na free	(2) + A	(3) - 1 G	(4) + Na	Qty
50	50	49	49	0.0
49	48	47	48.5	0.01
49	46	45.5	48.5	0.02
49	44	43.5	48 (kept)	0.03
	A	G		
49 kept 48.5	40.5 + 5 over 100 (3 x 1/10) 32 1 x 1 87	40.5 kept 40.5	48.5 kept 48	0.07 No Spectral shift .01

No spectral shift

16/5/74

Proteine estratte

1 | Estr. same as tharesia data — 94.5/0.025
2 | lipid exhact (HCl3:MeOH:H2O) · 2 ml — 93.5/0.03

3 | · 02 ml HC → 59.5/0.225
4 |

5 | · 05 ml B → 59/0.23
6 | 77/0.115
76.75/0.115

7 Bianchi 100/0.0

8 0.2 ml 60 µg | ovalbumina 92/0.0375 >> smalt. irrag.
9 0.4 ml 120 µg | 92/0.0375
10 0.6 ml 180 µg | 77.5/0.11

Na+ + K+ ATPase · 60'

1 HC Na 49.5/0.305
2 HC Na 50/0.30
3 B Na 77/0.112
4 B · Na :——
5 HC Na K+ 49/0.31
6 HC Na K+ ~~54/0.5~~ 49/0.31
7 B Na K+ 59.5/0.225
8 B Na K+ 60/0.22

~~Standard~~ Standard
9 Bianchi 100/0.0
10 1.1 mM 52/0.285
11 2.2 mM 36/0.44
12 3.3 mM 24.5/0.61

30'

5' incubation

13. Htc 83/0.08
14. Htc 87/0.06
15. B 94.5/0.025
16. B' 93.5/0.03
17 Blank II 100/0.0
18 0.1 μM 95.5/0.02
19 0.2 μM 91.5/0.0375
20 0.3 μM 89/0.05

AMP 8 hrs 3.5 mg
ATP 12 hrs 5 mg
(11 ml 7.1N NaCl and 0.05 M glycine) / 15 ml buffer

B D - B dialysed 20 hrs.
C D - C dialysed 20 hrs.

Bubin free ge dried $CHCl_3$: MeOH : H_2O 9:4:1

Bottom phase lipids
Middle - protein

MBP 165 | protein
MCP 165 |

MBL 165 | lipids
MCL 165 |

protein hydrolysate for ICRL

30/5/74 I MLH Hexane extracted lipids from whole brain homogenate (Monkey brain)

II MLCL $CHCl_3$: MeOH : H_2O 9:4:1 extracted lipids from whole monkey brain.

I = MLH. (1 mg in 3 ml roughly) Shaken in cyclo - particles removed made to 10 ml + 1 ml TNS.

II MLCL (5 mg - 6 ml roughly shaken made to 20 ml + 2 ml TNS

III MLCLTX - Half of above + 0.2 ml TTX

I 370 Excitation Max - 420 Emission Max.
6 x 1 range Blank: (19) Sample (44-45)

Blank ia.

I	I + A	I + G	I + Na	Qty
46	44	43	43	0.0
46	44	43	44	0.01
46	44	44	45	0.02
45.5	44	44	45.5	0.03
46	44.5	45	46	0.05

II 370 - 420 Blank [40.5] 4 x 1 range

II	II + A	II + G	II + Na	Qty
43.5	43.5	42	44	0.0
42	45.5 +1.5	42.00 1.5	44	0.01
43	50 +1.5	42.5 1.5	47.5	0.02
42.5	56 +1	43.5 1	54	0.03
42	62 +1.5	47.5 1.5	62.5	0.05

~~IV~~

III 370-420 4×1 range.

III	III + A	III + G	III + Na	Qty
36.5	34	35	36.5	0.0
36.5	36	35	37	0.01
35	43.5 +1.5	35.5	42	0.03
35	46.5 +1.5	38	46	0.02
35	51.5 +1.5	40	52.5	0.05

31/5/74

I. Hexane extract (CHCl3 dropped and suspended in water by shaking)

II Lecithin from Natraj (dried and shaken with water. 5 mg dissolved in 10ml hexane)

III Cholesterol – suspended like (I)

IV Lecithin + cholesterol. (II & III combined)

Blank 8×1 range 16

2×1 range 0.5

II

Range 2×1 360 - 440 Max excitations emission

II	II + A	II + G	II + Na	Qty
68	65	66	68	0.0
70	72.5	68	74	0.01
72	78.5	70	78	0.02
73	82	72.5	82.5	0.03

III 360 - 440 6 x 1 range. (Blank 5?)

No peak could be traced 360-440 readings

Calcein Scallerary?

III	III + A	III + G	III + Na	QL
43	38	37	37.5	0.0
42	38.5	37.5	37.5	0.01
43	40.5	40	38	0.02
43	42.5	42	38.5	0.03
43	44	44.5	38.5	0.05

IV 2 x 1 range peak excitation 360 Emisha peak at 440

IV	IV + A	IV + G	IV + Na	QL
52.5	54.5	54	53	0.0
52	57	54.5	55	0.01
53	60	56	58	0.02
53	64	56.5	61	0.03
52	67	57.5	65	0.05

I 8 x 1 range

Blank	I	I + A	I + Na	QL
15	45	43	43	0.0
15	44.5	45.5	45.5	0.01
15	45	48	47	0.02
15	45	50	48	0.03

TLC Solvent system

Hexane : ether : Acetic acid / neutral lipids
30 : 6 : 0.5

$CHCl_3$: MeOH : water / phospholipids
65 : 25 : 3.5

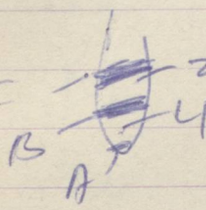
Protein estimation - 540 mμ.

1.	$CHCl_3$: MeOH : H_2O (Turbid) Bkm. exhaust.	65 / 0.195
2.	Hexane exhaust.	82.5 / 0.083
3.	C.S.F (2 ml)	87 / 0.06
4.	(I) Hexane extract	96 / 0.0175
5.	(II) Lecithin (turbid)	77 / 0.113
6.	(III) Cholesterol.	92.5 / 0.035
7.	Blank	100 / 0.0
8.	0.2 /	92.5 / 0.035 (wrong)
9.	0.4 / albumin 5 min	78 / 0.11
10	0.6 /	67.5 / 0.17.

4/6/74 Monkey brains - Hind leg analysed by ODA15 and normal 5 kgm each animal ht. killed - whole brain removed and kept in freezer for 24 hrs - frontal lobes for Cortex henceforth called NCX PCX and whole cerebellum NCB PCB

Roughly 8 gms of tissue homogenised in 30 ml of 8.5 % Sucrose in 10 mM Tris-HCl buffer 3000 rpm 1' pellet discarded. ~~Except~~ the top frothy layer & supt collected - spun at 10,000 rpm 10 min - pellet appearing brown bottom - suspended in 30 ml buffer

by gentle homogenisation in a Dounce .002" clearance homogeniser 10ml x layered over 10ml each of 40/28%. Rivers discontinuous density gradient. 85,000 rpm 2 hours (64,000 g) 30 min - Fraction round approximately



BPCX BNCX etc
CPCX CNCX
Kepl-sheared x at 0°.

5' incubation 6 hrs after extraction

(Crude and all 0.1 ml enzyme
0.1 ml H_2O instead
30' at 35°C shaken every 10'

(.1 ml Acetate buffer
.1 ml S/100 in 95%)

1	HCNCX	54.5/0.265	
2		50.5/0.295	
3	HCNCB	70/0.155	
4		64/0.195	
5	HCPCX	65/0.1875	
		61/0.215 * (5 times dilute)	
* 6.	HCPCX	Terminated after 2 hrs	
7	HCPCB	72.5/0.14	
8		72.5/0.14	
9	BNCX	86/0.065	
10		84.5/0.075	
11	BNCB	91/0.04	
12		91/0.04	
13	BPCX	92/0.035	
14		92/0.035	

5' incubation
1 ml (of 3 ml) aliquot
+ 0.5 ml Molybdate
+ 0.2 ml ANSA
+ 3 ml water
540 nm readings

15	BPCB	91	0.04
16		91	0.04
17	BNCX + A	87	0.06
18	BNCB + A	93.5	0.03
19	BPCX + A	93.5	0.03
20	BPCB + A	91	0.04
21	BNCX + S/100	89	0.05
22	BNCB + S/100	91.25	0.04
23	BPCX + S/100	91	0.04
24	BPCB + S/100	91	0.04
25	Blank	100.0	0.0
26	0.1 μmoles	99	0.005
27	0.2 μmoles	95	0.0225

+0.1 ml A (Rest balance in H2O)

0.1 ml S/100

Stds repeat

Blank 0.0

1 μmole 0.05

2 μmole 0.2

3 μmole 0.275

(Tris-HCl 8.5 pH 0.05 M longer in place of alkaline buffer)

C = 40.6 mg/2 ml

A = 72.6 mg/2 ml

D calculation

Long	Short		
1	0	I	BNCB
2	0	II	BNCX
3	0	III	CNCB
0	1	IV	CNCX
00	2	V	BPCB
00	3	VI	BPCX

Asp = 14.7 mg / 2 ml 0.05 M

Glu = 13.3 mg / 2 ml 0.05 M

Ranbal Labs

		Sample	Bu/th	ANS
BNCB	I	1	40	4
BNC x	II	1	40	4
BPCB	III	0.5	20	2
BPCx	IV	0.5	20	2
BNCB+ODAP	V	15 I + 0.2 ODAP(I) + 0.05 ODAP(II)		
BNCX+ODAP	VI	15 II + 0.2 ODAP(I) + 0.05 ODAP(II)		

ODAP (I) = 3.65 mg / ml

ODAP (II) = 25 mg / ml

I ANS Exatation 390 Emission 470

$I_{(6x1)}$ 46 Blank $_{(6x1)}$ = 7.5

I	I + A	I + Na	I + ODAP	Q Cy
45.5	43.5	41.5	44.5	0.0
43.5	43	43.5	44	0.01
43.5	44	45	44.5 (capt)	0.02
42.5	43	46	43.5	0.03

I	I + G	I + Glu	I + Asp	Q Cy
42.5	44.5	43.5	45	0.0
43.5	43.5	43.5	44	0.01
44	44.5	45	45.5	0.02
43.5	44.5	45.5	46.5	0.03
43.5	46	47	47	0.05

II	II +A	II +Na	II +ODAP	Q4
61	60.5	59	60	0.0
61	61.5	61	61	0.01
61	63	63.5	61	0.02
60	62.5	66	61	0.03
59.5	61.5	68	61	0.05
II	II +G	II +Glu	II +Hyp	
59	61.5	61	63.5	0.0
58.5	59.5	59.5	61	0.01
58.5	59	60	61.5	0.02
59.5	61	62.5	63.5	0.03
59	61.5	621	64.5	0.05

III	III +A	III +Na	III +ODAP	Q4
72.5	77	71	73	0.0
69.00	75	69.5	70	0.01
68.00	76	71.5	70	0.02
68.5	76.5	73.5	70.5	0.03
68.5	76.5	74	72	0.05
III	III +G	III +Glu	III +Hyp	
64.5	72	71	75	0.0
64.5	72	70.5	75	0.01
68.5	70.5	72	77	0.05

IV	IV +A	IV +Na	IV +Glu	6 x Average
40.0	38.5	39.5	40.0	0.0
40.0	40.5	41	40.5	0.01
40.0	41	42	42.5	0.02
39.5	42	41.5	43	0.05
+G	Loop	+Hyp	Loop	
41	41.5	42	42.5	.05

390-470 6x1 range

V	V+A	V+Bsp	V+Gln	
59.5	57.5	59.5	59.5	0.0
59.5	57	59	58	0.01
59.5	57.5	59.5	59	0.03
59.5 +G	56.5 +Na	60.5 kapl	60.5 kapl	0.05
62 kapl-	56.5 +Na	60.5 kapl-	60.5 kapr	0.05
62	56.5	60.5	60.5	0.1

G	G+A	G+Na	G+Gln	Qli
70.5	75.5	71.5	64.5	0.0
70	74.5	72	64.5	0.01
68.5	71.5	72.5	65	0.05
69	68.00	76	68	0.1
+G	+G	+Bsp	kapr	
71.5	67	78	68	0.05
73	69	77	68	0.1

6/6/74 (Night)

Na^+, K^+ ATPase.

Na^+			K^+		
HCNCB ①	BNCB ③		HCNCB ⑤	BNCB ⑦	
HCNCx ②	BNCx ④		HCNCx ⑥	BNCx ⑧	

(Supernatant) Colour of clear NO sediment (Separation)

0.06 ml all. 540 nm

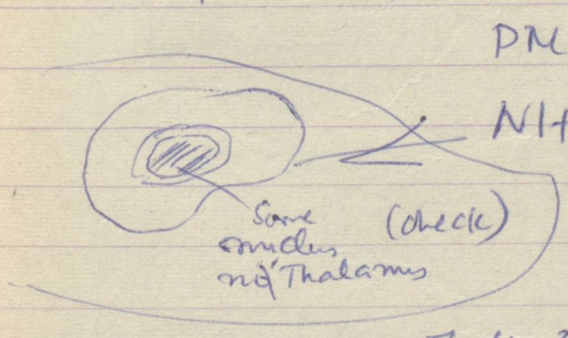
①	0.355 / 44	⑤	0.365 / 43
②	0.345 / 45	⑥	0.36 / 43.5
③	0.22 / 60	⑦	0.25 / 56
④	0.24 / 57.5	⑧	0.275 / 53

Standards

Blank	0 / 100
1 μMol	0.21 / 62
2 μMol	0.39 / 41
3 μMol	0.585 / 26

6/6/74

Merkley brain kept in 3/6/74
NMC Normal motor Cortex
PMC Paralysed motor Cortex
NHT : Normal 'Thalamus'



Homogenise approx 3 gms tissue in 30 ml 8.5% Sucrose in 10 mM 7.4 PH Tris. HCl buffer. 3500 rpm 1' Supt + Buffer Stuff - 10,000 rpm 10 mts - pellet suspended each (4 gms original) in 10 ml buffer Layered over 28% 40% discont Sucrose density gradient 30 mts 64000 g Microphor B. Cop. C.

III NHT II PMC I NMC

Crude III II I

BNHT	BPMC	BNMC
CNHT	CPMC	CNMC.

7/6/74 Oroleian 540 nm

·5M 0.5 ml Folin's
5 ml · Aprotinin · 1 ml Total Enzyme

1.	HNCB	0.03	47.5	/0.325
2.	HNCX	0.04	38	/0.42
3.	BNCB	0.05	73	/0.135
4.	BNCX	0.05	44	/0.36
5	I	0.02	72.5	/0.14
6	II	0.02	74	/0.13
7	III	0.04	72.5	/0.14
8	B I (BNHC)	0.05	86	/0.065
9.	B II (BNHC)	0.05	71	/0.15
10	B III (BNHT)	0.05	75	/0.125

In previously recorded.

Pancytol NTPase activity much less.

Blank		100 /0.0
0.2 (60)		93.5 /0.03
0.4 (120)		87 /0.06
0.6 (180)		83 /0.08

0 Valban 300 µg/ml

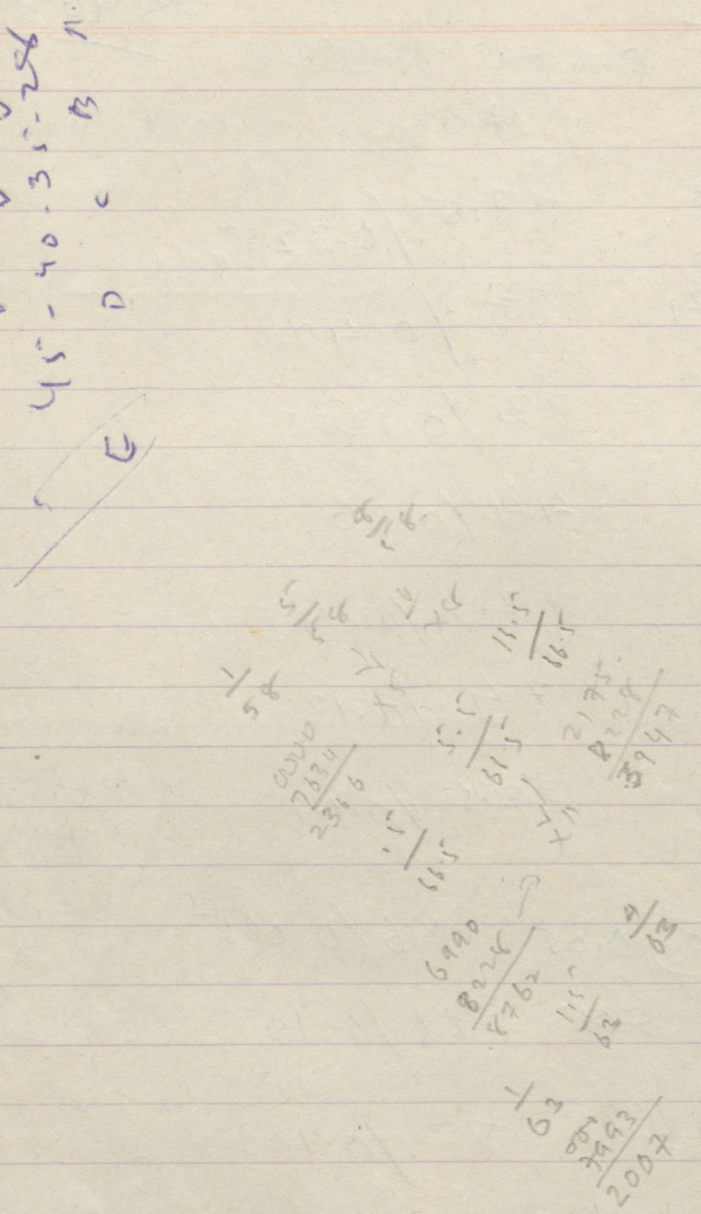
→

I - B I
II - B II
III - B III

IV B I + ODAP
V B III + ODAP

ODAP 7 mg / 2 ml.

FTS next w/-


45 - 40 - 35 - 25
D
E

Pfeiffer

ODAP 0.8 ml to 20 ml. of Buffer

TNS 1 ml to 100 ml + 0.5 ml Sample.

370 Excitation Max 430 Emission Max.

Blank 3 (4x1 rays) Sample I (62 (4x1 rays)

Blank 3.

I	I + A	I + Glu	I + Na	Alg
65	59	58	60	0.0
65	62	59	62	0.01
65	66	63	67	0.02
65	69	66	72	0.03
65	69.5	67	75	0.05

I	I + K	I + Asp	I + G	
66	66.5	63	63.0	0.0
66	67	64	62.5	0.01
66	72	64.5	62.5	0.02
66	83	67	64	0.05

4x1 rays

II	II + A	II + Glu	II + Na	Alg
77	80	71	75	0.0
77	82.5	73	82	0.01
77	86	76	87	0.02
77	89	83	94	0.05

II	II + K	II + Asp	II + G	Alg
69	70	69	67	0.0
69	72	69	68	0.01
69	76	72	67	0.02
69	87	73	68	0.05

III 5 x 1 range

III	III + A	III + Glu	III + Na	Q[Ca]
54.5	52.5	54	53	0.0
54.5	55	54.5	56	0.01
54.5	59	57.5	59	0.02
54.5	62.5	61	66	0.05

III	III + k	III + Asp	III + Ca	
55	54.5	55	54.5	0.0
55	57.5	56	56	0.01
55	59.5	57	56	0.02
55	69	61	56	0.05

IV 4 x 1 range.

IV	IV + A	IV + Glu	IV + Na	Q[Ca]
60	57	62	59	0.0
60	60	62	62	0.01
60	62	64.5	63	0.02
60	64	67.5	67	0.05
+K	kept	kept	kept	
62.5	61 ?	65.5 ?	67	0.02
69	62 ?	66 ?	67	0.05

V 3 x 1 range

V	V + A	V + Glu	V + Na	Q[Ca]
54	55	54	54	0.0
54	55	54	55.0	0.01
54	56.5	56	56	0.02
54	57	58	58.5	0.05
+K	kept	kept	kept	
57	57	58	58.5	0.02
64	57	58	58.5	0.05

a little
Serotonin - Blue colour my
world
Adrenaline: Yellow milkshakes
- Solitary confinement

8/6/74

37°C 540 nm

A : 0.1 ml S/100 : 0.2 ml.

whole blood developed. (Centrifuged & clear supt Color Checked)

5' nucleohdase 30' assay. Nat Kt ATPase 60'

		OD		Na+	K+, Na+	
1	HNMC	0.1	HNMC	17	0.105	29 0.118
2	H PMC	0.135	HPMC	18	0.102	30 0.118
3	HNHT	0.0725	H NHT	19	0.075	31 0.095
4	BNMC	0.05	BNMC	20	0.08	32 0.13
5	BPMC	0.07	BPMC	21	0.075	33 0.11
6	BNHT	0.0725	BNHT	22	0.1	34 0.12
7	BNMCA	0.04	BNMCA	23	0.015	35 0.01 *
8	BPMCA	0.055	BPMCA	24	0.01	36 0.055
9	BNHTA	0.07	BNHTA	25	0.0425	37 0.043
10	BNMC S/100	0.055	BNMC S/100	26	0.0525	38 0.148
11	BPMC S/100	0.07	BPMC S/100	27	0.0525	39 0.145
12	BNHT S/100	0.08	BNHT S/100	28	0.125	40 (broken)
13	Blank	0.0	Blank	41	0.0 ←	
14	1 μM	0.27	1 μM	42	0.33	
15	2 μM	0.495	2 μM	43	0.66	
16	3 μM	0.75	3 μM	44	1.2 X? *	

(0.05 ml Sample on each)

AMP- 16 mg 16 hrs

0.5 ml (.1 ml $MgCl_2$, in 0.5 pH .05M Tris HCl buffer)

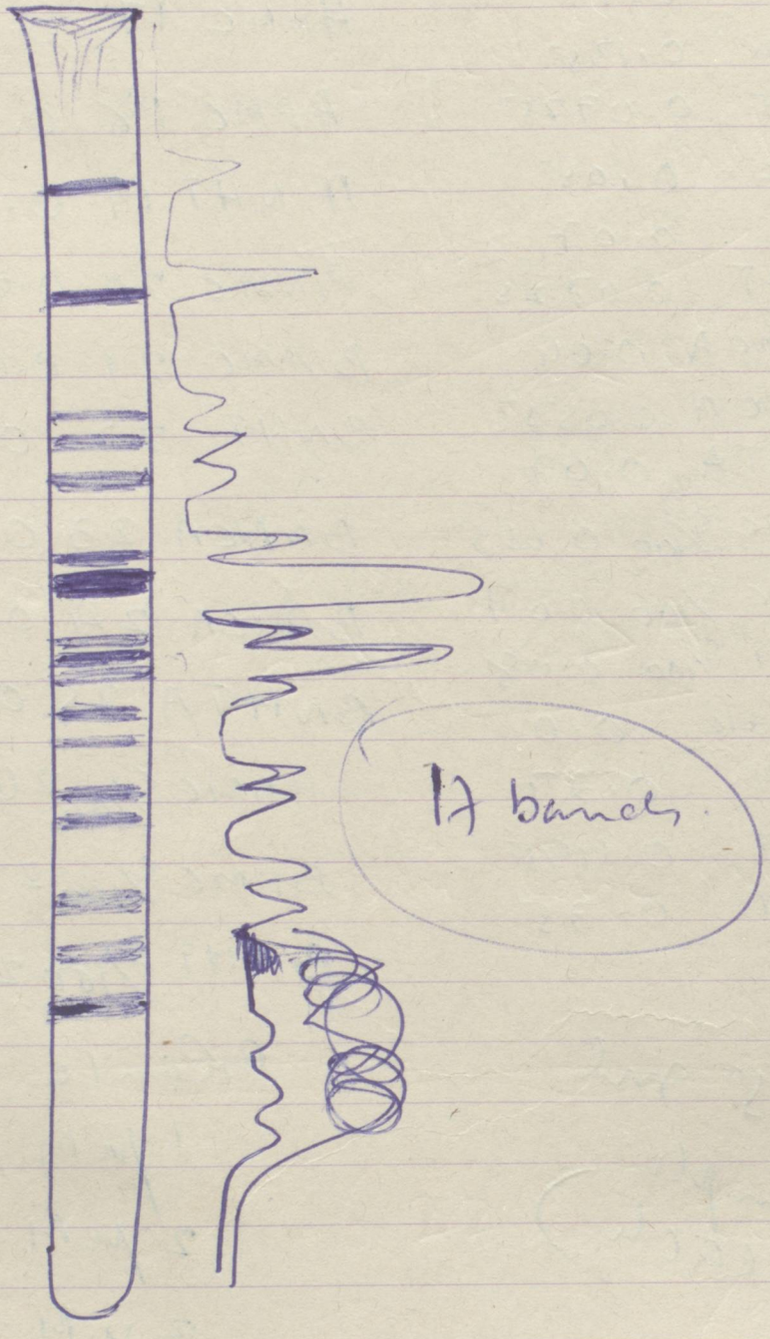
Perchloric 2.5 ml 5% TCA

ATP 14 mg 28 hrs

(EDTA 32×10^{-4} M ultimate concentration) 0.5 ml + .16 ml Solution D)

0.05 ml Sample each.

RB



C-M extract proteins

		band
1	BNMC	9
2	BPMC	10
3	BNHT	11
4	CNMC	12
5	CPMC	13
6	CNHT	14

Earlier

1	I
2	II
3	III
4	IV
5	V
6	VI

Anhäufung Reddy.

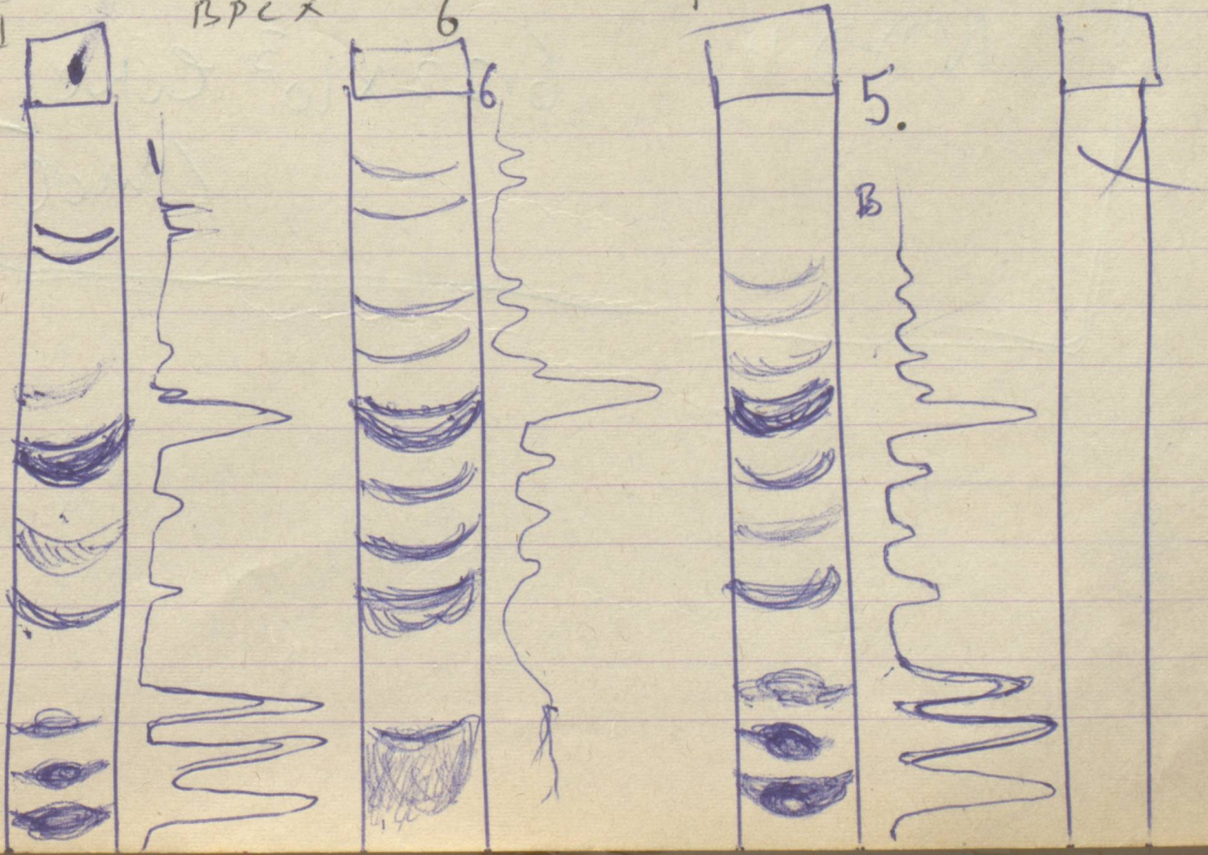
Electrophoresis

		Tube No
2	BPMC	7
1	BNMC	8
4	CNMC	9, 10*
I	BNCB	1
II	BNCX	2
III	CNCB	3
IV	CNCX	4
V	BPCB	5
VI	BPCX	6

SDS - polyacrylamide Gel electrophoresis

5 mA/tube

1 mg protein · 1.5 ml 4% SDS Tris buffer.



A cholinesterase

Rate : moles/litre per min-

: D absorbance/min

$$\frac{}{} = \frac{5.74 \times 10^{-4} \Delta A}{C_0}$$

C_0 = tissue Conc mg/ml

NAD+ : 6.22×10^3 litre $/mol^{-1}$

5/8/74

Procedures

(Brain kept frozen for two weeks. F presumptad).

Tissue - motor Cortical regions about 30 gms
Homogenised FHC in 120 ml 8% Sucrose in 10 mM Tris
HCl (PH 7.4) 4 tubes, Centrifuge 1000 g 1'
Supt Spun 10,000 g 10' - pellet
Sans brownish both parts + buffer to 38 ml volume.
Homogenise & keep for 2 hrs at 0°C
Spin 30-45' 64,000 g 40/28 ch.
Interphase Collected. FMB
Stored in freeze in Sucrose.

0-4°C

If fresh

Adenylate cyclase

1.6 mM ATP (α^{32}P 4 in Amersham Columns stock)
3.6 mM $MgCl_2$
7.3 mM Theophylline
0.1 % albumin.
0.36 mM Glycyl glycine.
0.18 mM $MgSO_4$
30 mM Tris HCl. PH 7.4
Final assay volume 55 µl Contain 10 µl
Adenylate cyclase preparation 30-70 µg protein.
Incubation started by addition of enzyme 15' 37°C
Terminated by boiling

J. Ramachandran

step up to 1 ml
1 ml each
2 ml prep

5/8/74 Frozen brains - motor cortex regions 10 gms
120 ml (3180) mg/ml + sucrose to 8% PH 7.4 homogenised
4 tubes 1000 g 1'
Supt 10,000 RPM 10'
Pellet + buffer to 30 ml vol
homogenised - kept - kept overnight at 0°
Spun 45' 40/28 interphase
& kept 2 sets. collected
BM 58 CM 58

GK/74 unchalyted BM5.

ANS
TNS | 0.3 mg/ml. Buffer Tris 10mM PH 7.0

Solution in buffer.

Na	0.2M		
G.	Gaba	0.2M	22 mg/ml.
A	Ach	0.2M.	36.3 mg/ml
Asp	DSparkle	0.2M	
S-	Serotonin	0.05M	19.37 mg/ml

Memb 0.1 ml +2.4 ml buffer
add ANS TNS varying Conc

ANS
TNS | 0.1 ml +2.4 ml buffer. add Memb.

Treatment of membrane | Memb 0.5 ml + X

S	G	Na	Asp	Ach	Free
X = 0.05 S +0.25 buffer	0.3 G	0.3 Na	0.3 Asp	0.3 Ach	0.3 buffer.

I TNS 370 - 430

(I) Free	(II) Ach	(III) Na	(IV) S	Add Memb Free 1	Ach 2	Na 3	S 4
-	forgot.	-	13	15.5			0.0
61 (6x1)	66 (6x1)	57 (6x1)	65 (6x1)				0.02
67 (5x1)	74 (5x1)	68 (5x1)	69 (5x1)				0.03
66 (4x1)	77 (4x1)	71 (4x1)	72 (4x1)				0.05
71 (3x1)	85 (3x1)	85 (3x1)	75 (3x1)				0.1
54 (2x1)	66 (2x1)	63 (2x1)	56 (2x1)				0.1

Spectra 7 "DNS-Chol": in methanol

(conversion concentration) (for spectra only)

DNS · (5×1)

330 Excitation max 460 emission max

emission 460		Excitation 330	
excitation spectr		emission spectr	
220	3	300	0.5
230	5.5	310	1
240	17	320	15.5
250	31	330	76
260	32	340	45
270	26	350	46
280	35.5	360	7
290	75 (5×1)	370	8
300	74 (4×1)	380	9
310	60 (3×1)	390	10.5
320	74 (3×1)	400	13.5
330	79 (3×1)	410	19
340	71 (3×1)	420	30
350	55 (3×1)	430	48
360	60 (4×1)	440	64
370	66 (5×1)	450	80
380	29	460	87
390	14.5	470	88
400	9	480	80
410	5	490	68
420	3	500	55
430	3	510	42.5
440	12	520	33.5
450	66 (4×1)	530	24
460	60 (3×1)	540	17.5
		550	13
		560	10
		570	7

DNS-Chol Emp. I (5×1) 360 – 470

Em. 470 excitation spectr		Excitation 360 emission spectrum	
220	2.5	300	2.5
230	2.5	310	2.5
240	3	320	2.5
50	3	330	2.5
60	3.5	340	2.5
70	9	350	3
80	16	360	3
90	17.5	370	3
300	13	380	2.5
10	11	390	3.5
20	11.5	400	6
30	15	410	12.5
40	32	420	25
50	62	430	44
60	95	440	65
70	91	450	82
80	54	460	92
90	26.5	470	95
400	11	480	88.5
10	4.5	490	75
20	3	500	60
30	3	510	47
40	2.5	20	35
50	3	30	25
60	4	40	17
70	4.5	50	12.5
80	2.5	60	9.5
90	2.5	70	7.5
		80	5.5
		590	4
		600	3

(Conc. corresponds to last reading on pg 157)

6/8/74 (unheated membrane)

Free + TNS probe

2x1 range. (Membrane unheated)

Excitation 370 to emission 430

Emission 430	(2x1) range) Excitation 370
Excitation probe	Emission probe
250 - 6	400 - 40
260 - 8	410 - 46.5
270 - 14	420 - 52
280 - 24.5	430 - 54
290 - 28	440 - 51
300 - 30.5	450 - 45
310 - 41	460 - 34
320 - 53	470 - 32.5
330 - 59	480 - 25
340 - 50.5	490 - 19.5
350 - 51	500 - 15
360 - 55	
370 - 54	
380 - 34.5	
390 - 37.5	
400 - 33.5	
410 - 28.5	
420 - 24	
430 - 18.5	
440 - 15	
450 - 11.5	

Blank TNS (unheated)

6x1 range.

Excitation to Emission 410

		Excit 360
220 -		Emission probe
230 -		350 40
240 -		360 avalue
250	2.5	370 67
260	3	380 19
270	6	390 24
280	13.5	400 28
290	21.5	410 28.5
300	26	420 24.5
310	21	430 20
320	13	440 18
330	14	450 17.5
340	17	460 17.5
350	25	470 16
360	30	480 18
370	24.5	490 18
380	7	500 16
390	5	510 14
400	70	520 12.5
410	3x1=40	530 11
420	95	140 9
430	4	550 7
440	1	560 6
450	1	

Ach treated membrane TNS

Emission 430

Excit	Reading
250	6
260	9
270	16.5
280	30
290	34
300	38.5
310	52.5
320	70
330	76
340	64
350	65
360	69
370	67
380	46
390	20
400	19

Excitation 330

Emission	Reading
310 -	10
370 -	9
360 -	13
390 -	25
400 -	40.5
410 -	56.5
420 -	70
430 -	75
440 -	73
450 -	66
460 -	54
470 -	45
480 -	36
490 -	26
500 -	19

Na

P

S

treated

probe

similar to

Ach treated

360-430 TNS

(4×1) mgs

I	II	III	IV	0.3 mg/ml
Fill	Ach .3ml .2M	Na .3ml .2M	S .05ml .05M	TNS & 2.5 ml added
4	4	4	4	0.0
19	21	20	22	+0.01
32	38	34.5	33	+0.02
46	50	46.5	44	+0.03
59	67	63	59	+0.03
80	67	72.5	77	+0.05
60	64	61	60	+0.1

ANS Blank

Emm 510

Exch	
250	2.5
260	3.5
270	5.5
280	7.5
290	8
300	8.5
310	8
320	10.5
330	15
340	20
350	23.5
360	25.5
370	26.5
380	24.5
390	20
400	14
410	7
420	3.5
430	2.5
440	1.5

450 0.5

460 0.5

Exch 370

Emm

370	45
390	2.5
400	1.5
410	2
420	4.5
430	5
440	4
450	6
460	9.5
470	14
480	19
490	23
500	25.5
510	26.5
520	25.5
530	23
540	20
550	17.5

ANS

Blank

Same

340 - 470 (5"x1) rmp. ANS

I	II	III	IV	Mercury (after added
Free	Ach	Na	Serum	
5" · 5"	5"-5"	5" · 5"	5" · 5"	0.0
11" · 5"	16 · 5"	15" · 5"	16	+ 0.02
31 (4x1)	34	32	30	+ 0.03
5" 4	62.5	5" 9	52	+ 0.05"
57 (4x1)	61.5 (4x1)	58 (4x1)	52 (4x1)	+ 0.1

4x1 rmp 340 - 470 ANS.

I	II	III	IV	Add ANS
Free	Ach	Na	Serum	
7	8	5"-5"	8	0.0
10.5"	10	9.5"	10.5	+ 0.01
15" · 5	16 · 5	12 · 5"	14	+ 0.02
23	26	19	19 · 5"	+ 0.03
30 · 5"	33	25"	25	+ 0.04
44	45 · 5"	34	35"	+ 0.1

7/8/74 5/8/74 Overpn

360-430 TNS 0.3mg/ml. dilution as previous. 6/8/74

I Asp (4x1)	II GABA	TNS ADD	III Asp (4x1)	IV GABA	Membs added
10	10	0.0	4.5	4.5	0.0
20	16	+0.01	16.5	13.5	+0.02
38	27.5	+0.02	36.5	26.5	+0.03
64	41	+0.03	80	48.5	+0.05
87	55	+0.04	84(3x1)	68(4x1) 48(3x1)	+0.1
73(3x1)	86(4x1) 45(3x1)	+0.1	74(2x1)	71(3x1) 37(2x1)	+0.1

3x1 range. 390-470

I Asp	II GABA	ANS ADD	III Asp	IV GABA	Membs Added
3	3.5	0.0	1.5	1.5	0.0
5.5	5	+0.01	5	3.5	+0.02
9.5	7	+0.02	10	7	+0.03
15.5	10	+0.03	20	13	+0.05
27	16	+0.1	40.5	23	+0.1

Glu 0.1 M

Ala 0.1 M

Pro 0.1 M

SHel - 0.05 M

NHel - 0.05 M

S, N 0.05 + .15 ml buffer

Rest 0.2 ml

Free 0.2 ml buffer.

7/8/74 370 - 430 TNS.

	I Free	II Strl	III Nitrl	IV Oro.	Mems added
(6x1)	14.5	12	11	11	0.0
(6x1)	65	92	93	66	+0.02
(4x1)	35	64	66	38	+0.03
(3x1)	34	73	77	37.5	+0.05
(2x1)	32	83	83	35	+0.1

TNS Blank Specks (6x1) rpm

Emission 410 Excitation Specks	Excit 300	Excit 360
250 — 3	310 — 22	370 — 14
60 — 4	320 — 4	80 — 15
70 — 6.5	30 — 4	90 — 16
80 — 12	40 — 5	400 — 19
90 — 25	50 — 4	10 — 17
300 — 26	60 — 3.5	20 — 13.5
10 — 17	70 — 11	30 — 13
20 — 11	80 — 18.5	40 — 13
30 — 10	90 — 24.5	50 — 13.5
40 — 13	400 — 26.5	60 — 14
50 — 17	10 — 24.5	70 — 14.5
60 — 24	20 — 21	80 — 14
70 — 19	30 — 17	90 — 13.5
80 — 5	40 — 14	500 — 12.5
90 — 6	50 — 12.5	10 —
400 —	60 — 12	
	70 — 11.5	
	80 — 11.5	
	90 — 10.5	
	500 — 9.5	
	510 —	
	1	

II Mems (Serotrin treated) + TNS (2x1) rpm

Emission 425 Excit	Excit 330 emit	Excit 360 emit
250 — 3.5	340 —	380 55
60 — 5.5	50 — 65	90 35
70 — 8	60 — 17	400 50
80 — 13.5	70 — 14	10 65
90 — 19.5	80 — 19	20 73
300 — 25.5	90 — 34	30 74.5
10 — 43	400 — 55	40 69.5
20 — 72	10 — 75	50 60
30 — 80	20 — 88	60 50
40 — 67	30 — 91	70 41
50 — 67	40 — 86	80 31.5
60 — 75	50 — 72	90 23.5
70 — 75.5	60 — 61	500 17.5
80 — 55	70 — 49	
90 — 32	80 — 37	
400 — 22	90 — 27	
	500 — 20	

N, Oro Strl

Free Mems + TNS

Emission 430		
250 — 3.5	310 — 25.5	370 — 37
60 — 5.5	320 — 32.5	80 — 23
70 — 10.5	30 — 35.5	90 — 16
80 — 17	40 — 29.5	400 — 13.5
90 — 19	50 — 21.5	
300 — 19.5	60 — 31.5	

Excitation 330

360 — 8	440 — 34.5
70 — 7	50 — 30.5
80 — 9	60 — 26
90 — 13.5	70 — 22
400 — 20.5	80 — 17
10 — 28	90 — 13.5
20 — 33.5	500 — 10
30 — 35.5	

Excitation 360

390	20
400	23.5
10	27.5
20	31
30	31.5
40	30
50	26.5
60	23
70	19
80	15.5
90	10.5
500	9

[330-430] TNS

	Free I	SHd II	NHd III	Pro IV	Add TNS
4x1	12.5	13.5	12.5	14	0.0
4x1	18.5	27.5	27	22.5	+ 0.01
4x1	30	53	54	34	+0.02
4x1	46	62	91	51	+0.03
3x1	61.5 (4x1) 34 (3x1)	63 (3x1)	70 (3x1)	68 (4x1) 37 (3x1)	+ 0.04
3x1	50.5	93	102	57	+0.1

[330-430] TNS

	I Gly	II Ca++	III K+	IV (residual) Gln	Memb added
6x1	9.5	9.5	9.5	10.5 ←	0.0
6x1	59.5	overflow 57.5 (5x1)	57	45	+0.02
	39 (4x1)	96 (4x1)	39 (4x1)	32 (4x1)	+0.03
	37 (3x1) 20 (2x1)	overflow 58 (2x1)	42 (3x1) 23 (2x1)	35 (3x1) 18.5 (2x1)	+0.05
	36 (2x1) 19 (1x1)	overflow 70 (1x1)	45 (2x1)	41 (2x1)	+0.1

330-430 TNS

	I Gly	II Ca++	III K+	IV Gln	TNS added
5x1	17.5	16.5	17.5	11.5	0.0
5x1	37.5	77	39.5	27	+ 0.01
5x1 4x1	71 38.5	overflow 92	81 44	52 27.5	+0.02
4x1 3x1	57 31	overflow 77	65 36	43 23	+0.03
3x1 2x1	42.5 23 27	overflow 62 63	49 27 29	32 17 20 (2x1)	+ 0.05 + 0.1

ANS Blank (7×1)

Emission 510

250	4·5
60	7
70	10
80	12
90	12
300	10
10	11
20	15
30	21
40	30
50	35
60	38·5
70	38·5
80	36
90	29·5
400	21
10	11
20	5
30	4

ANS (7×1) blank

Exam 365

390	2·5
400	3
10	5·5
20	6
30	7
40	6
50	10
60	15·5
70	22
80	30·5
90	37
500	41
10	42
20	40·5
30	37
40	32
50	28
60	23

390 - 470 DND.

Serum treated Memb + ANS (3×1) rate.

Emm 470

250	5
60	6·5
70	9
80	17
90	24·5
300	25
10	24·5
20	26
30	31
40	43
50	53·5
60	62·5
70	73
80	82·5
40	84
400	70
10	42
20	24
30	15

Exam 390

410	90
20	27
30	33
40	46
50	59
60	69
70	78
80	75
90	67
500	57
10	46
20	36
30	28

Range	I Free	II Silica	III Nitre	IV Pro	Memb added
6×1	10	10	9·5	10··	0·0
6×1	26	44	46	29	+0·02
6×1	52·5	avalue	avalue	59	+0·03
5×1	28·5	55	57	32	
5×1	50	avalue	avalue	57	+0·05
4×1	27	61	62	31	

ANS added

5×1	13	15	13·5	15	0·0
	19	28	26	20·5	+0·01
	27	49·5	47·5	28·5	+0·02
	26·5	70	74	41	+0·03
5×1	53	avalue	avalue	56	+0·05
4×1	29	60	59	30·5	
4×1	41	84	83	43	+0·1

390 - 470 mμ BNS

I Gly	II Ca^{++}	III K^+	IV Glu	Mimb added	Range
10	10	10	10	0.0	6x1
31.5	45	31	21	+0.02	6x1
61	96	60	39.5	+0.03	6x1
56	avalov	59	35	+0.05	5x1
30	57	32	19.5		4x1
53	avalov	60	35	+0.1	4x1
29	63	32.5	20		3x1

			BNS added (TURBID)	~~ATP~~ range
15	13	14	6	0.0	5x1
20.5	22	20	8.5	+0.01	5x1
28.5	38	29.5	14	+0.02	5x1
40	57.5	42.5	20.5	+0.03	5x1
53	76.5	57	—	+0.04	5x1

8/8 1x1 Adenylate cyclase

1.6 mM ATP
3.6 mM $MgCl_2$
7.3 mM Theophylline
0.1 % Albumin
0.36 mM Gly-Gly
0.18 mM $MgSO_4$
30 mM Tris HCl pH 7.4

1/5 total assay tube preparation

Blue NaF = ATP Case

15' 37°C
Terminate 5% TCA
3 ml //

~~Adenylate cyclase~~ Adenylate cyclase

[Total vol 1 ml + 3 ml TCA + 2 ml membrane]

		ng cAMP formed (per ml)	
①	B8-1	520	540-77
②	B8-2	560	
③	H-8-1	670	590-77
④	H8-2	510	
⑤	B5-1	560	575-77
⑥	B5-2	630	
⑦	H5-1	415	388-77
⑧	H5-2	370	
⑨	Blank	B5 0.2 after acid added	77

ng cAMP formed (per ml) to calculate pmol/mg protein/min

B8 = 23000 / 9850

H8 = 9850

B5 = 28000 / 3490

H5 = 3490

9/8/74

Acetylcholinesterase

NO DTNB as Blank (5' in DTNB) 8 on 5 min incubation

		After 81		Protein
1	H8	31/0.505	7.5/1.25	
2	H8	29/0.54	7.5/1.25	0 100/0.0
3	B8	55/0.26	8.5/1.15	60µg 85.5/0.067
4	B8	56/0.25	8.75/1.125	120µg 77.5/0.11
5	H5	30/0.52	6.5/1.45	180µg 64/0.195
6	H5	33/0.48	8/1.2	H8.02 55/0.26
7	B5	59/0.23	10/1.0	H5.02 39/0.41
8	B5	60/0.22	10/1.0	B8.04/63/0.20
9	Blank No substrate	93.5/0.03	85/0.07	B5.04 65/0.185
10	No enzyme	89/0.05	90/0.045	mg/ml
11	No DTNB	100/0.0	100/0.0	H8 - 13
				H5 - 20
				B8 - 5
				B5 - 4.65

10/8/74

DNS - choc Component Aqueous Gradn
[0.3 ml W 7.5 ml]

(3x 1/10) range

(No conc adjustment)

Emission 495 (max)

membrane introduced to the above electrode

Blank

Exctatn 340

Emission 495	
250	5
60	10.5
70	16.5
80	26.5
90	40
300	50
10	61.5
20	74
30	88
40	94.5
50	82
60	59.5
70	42
80	21.5
90	12
400	6.5
10	3.5

Exctatn 340	
300	2
10	2
20	2
30	2
40	2.5
50	2.5
60	2.5
70	2.5
80	2.5
90	2.5
400	2.5
10	2.5
20	4
30	7
40	14
50	28
60	45
70	66
80	84
90	94
500	95
10	87.5
20	78
30	62
40	50
50	38

I

Mcnts added

Emsn 495	
250	4
60	7.5
70	11.5
80	19
90	30
300	39
10	49
20	60
30	73
40	81
50	73
60	55
70	37
80	21
90	11.5
400	6.5
410	

Emsn spectn Exct 340	
300	2
10	2
20	2
30	15
40	ovflo 100
50	80
60	5.5
70	3
80	3,3
90	2.5
400	3
10	3.5
20	6.5
30	12
40	24
50	39
60	56
70	72.5
80	80
90	80
500	
10	74
20	65
30	—

(0.15 ml in 10 ml)

II

Na Fraction 3x1/10

Emsn 500

300	40	250	3.5
310	50	60	7
320	60	70	11
30	72	80	18
40	80	90	29
50	73		
60	54		
70	37		
80	20		
90	11		
400	6		
10	4		
20	2.5		
30	2.5		

Exctn 340	
300	2
10	2
20	2
30	17
40	ovflo
50	76

360	5	520	64
70	3	530	53
80	2.5	540	43
90	2.5		
400	2.5		
10	2.5		
20	3.5		
30	6.5		
40	13		
50	23		
60	39		
70	55		
80	71		
90	81		
500	74		
510			

Ach Fraction 3x1/10

Emsn 500nm	
250	4
60	8
70	12
80	20
90	32
300	42
10	54
20	65
30	77
40	85
50	76
60	56
70	39
80	21
90	12
400	6.5
10	
420	

Exctn 340	
300	2
10	2
20	2
30	18
40	ovflo 100
50	85
60	8
70	3
80	2.5
90	2.5
400	2.5
10	2.5
20	4
30	7
40	13
50	24.5
60	41.5
70	59
80	75
90	84
500	85
10	78
20	69
30	55
40	44

550	34		
560	27		
570	18		

DRS.chol 3x1/10

Gweli 3. Serohmi Excitation 340

Worked

500 emission

250	2	360	53.5	300	2	410	2.5
60	3	70	36	310	2	20	3.5
70	3.5	80	19.5	20	2	30	6
80	4.0	90	12	30	21.5	40	12
90	5.5	400	6.5	40	99	50	23
300	8.5	410	3.5	50	74	60	37.5
10	20.5			60	7.5	70	53.5
20	47.5			70	3	80	69
30	66			80	2.5	90	75.5
40	77.5			90	2.5	500	76
50	70			400	2.5		

510	70		
20	61.5		
30	50		
40	36.5		
50	31.5		
60	24		
70	17		
80	11		

12/8/74 Drop in DRS chol 1/10 of 10/8/74 in H_2O + Meals 1½ tms Reagent 0.45 ml in 10 ml

[A] Specific Same as above. 2x1 range

[B] Drop in diluted by Longon from 10 tms of 0.45 ml meals to 10 ml in Longon (Longon adventist Zaria)

Blank 6x1 range

495 emission

250	10.5	340	7	260	22	400	63
60	6.5	50	4	70	17	410	57
70	6	60	6.5	80	23	420	53
80	10.5	70	5.5	90	38.5	430	51
90	23	80	5	300	57	440	52
300	38.5	90	6	10	73	50	58
10	52	400	7	20	83	60	62
20	59.5	10	7	30	82	70	78.5
30	56.5	20	7.5	40	68	80	84
40	43	30	8.5	50	55	90	85.5
50	29	40	12	60	43.5	500	82
60	17	50	19	70	37.5	10	74
70	10	60	30	80	32	20	63
80	5	70	42.5	90	29	530	53
90	3	80	53.5	400	27		
400	2	90	58.5	10	26		
		500	58				
		10	53.5				
		20	47				
		30	37.5				
		40	29.5				
		50	23				
		560	17				

Excitation 320

540	43		
50	35		
60	29		
70	22		
580	17		

13/8/74 Freshly lulled female Wonley Grain quota Cotix Isolchi

H13	PND 13	LS 13	B 13	B 38,5° DIAL	C 8,5° DIAL	H 13	B 13	NO Reagent
0.36/43.5	1/10	0.36/43.5	0.37/42.5	0.19/65	0.38/42	0.325/47.5	0.41/39	0.21/44.5

5' machine no Reagent after 5' heated in DTNB 5' NO DTNB Blank

0.79/16.5	1.95/~~4.0~~ 2/10	0.875/13.5	1/10	0.72/18	0.75/17.5	0.85/14	1.1/8	0.245/57

Antony SDS. Polyacrylamide

10× buffer	1 ml	
20% SDS	0.5 ml	
Acryl Gel soln.	2 ml	2.7 ml per tube
Ammon. persulfate	15 mg/ml	soln 1.0 ml
TEMED	2.5 µl	

add water to 10 ml.

12/6/71

I 0.75 TNS (0.3 mg/ml) + 15 ml buffer. Blank

II 0.5 ml B(5 µg) DIAL + 12.5 ml I | Dilution not exact!

.01 ml 0.2M salt & 2.5 ml. ~0.8 mM | PB

TNS

①	②	3	Li 4
Blank (9×1)	Memb + TNS	Cs (5×1)	Li

Blank I TNS

Emission 480	Exct 330
250 6	350 6
60 9	60 6.5
70 14.5	70 20
80 23	80 30
90 33.5	90 33
300 43	400 37
10 51.5	10 39.5
20 59	20 39
30 63 ←	30 41
40 59	40 45
50 60	50 51
60 60.5 ←	60 56.5
70 51	70 61.5
80 32	80 63.5 ←
90 16	90 61.5
400 10.5	500 57
10 11	10 51.5
20 6	20 44
30 4	30 37
40 3.5	40 30.5
	50 26
	60 21
	70 16
	80 10.5
	90 6
	600 3.5

(9×1 range)

II Memb + TNS

Emission 430	Exct 330
250 6.5	360 8
60 11	70 8.5
70 21.5	80 13
80 41.5	90 25
90 42	400 43
300 42.5	410 61
10 56	420 75
20 74	430 78 ←
30 79.5 ←	40 76
40 67	50 67
50 69	60 56.5
60 74 ←	70 47
70 71.5	80 36.5
80 47	90 27.5
90 26	500 20.5
400 17	10 15
	20 11
	30 8
	40 6
	550 4

(5×1) rge.

Cs Cl treated

Emission 430	Exct 330
250 7	360 7.5
60 11.5	70 8
70 22	80 14
80 42	90 25
90 43	400 42.5
300 45	10 62
10 59	20 76
20 78.5	30 85
30 85	40 88
40 72	50 70
50 73	60 60
60 78.5	70 49
70 75	80 37.5
80 50	90 28
90 27	500 21.5
400 16	10 15.5
	20 11.5
	30 8
	40 6

5×1 rge.

Li treated

Emission 430

Exct

250	8
60	13
70	27
80	50
90	50
300	53
10	71.5
20	78
30	78.5
40	94 - 320
50	98.5 - 330
60	81.5 - 340
70	82 - 350
80	88 360
90	84 370
400	55 - 380

29 18 390 410

Input to table

Curves

4 → 1

3 → 4

1 → 3

×2

3x1

5x1

TNS + N2 treated Mamb. 5x1

Emstm 430		Exuli 330	
250	6·5	360	8
60	11·5	70	8·5
70	22·5	80	14
80	42·5	90	28
90	45	400	47
300	46·5	10	68
10	61	20	83·5
20	80·5	30	88
30	88	40	85
40	74	50	73·5
50	74·5	60	63
60	60·5	70	51·5
70	77	80	40
80	51	90	29
90	27	500	22
400	17	10	16
		20	12
		30	8
		40	6
		50	4
		560	3

5x1

K+ treated Mamb + TNS 5x1 rxn

Emstm 430		Exuli 330	
250	7	360	8
60	12	370	8·5
70	22·5	80	14
80	43·5	90	28
90	44·5	400	48
300	46	10	69
10	84	20	84
20	84	30	89
30	90	40	86
40	75·5	50	75
50	77	60	63·5
60	63·5	70	52·5
70	79	80	41
80	54	90	30·5
90	28	500	23
400	17·5	10	16·5
		20	12
		30	8·5
		40	6
		50	4·5
		560	3·5

5x1 rxn

Ca++ treated Mamb + TNS 3x1 rxn

Emm 430		Exuli 330	
250	5	360	3
60	9	70	4
70	17	80	9
80	30	90	21
90	31·5	400	38
300	39·5	10	57·5
10	57	20	73
20	76	30	76·5
30	79	40	77
40	67	50	64
50	64	60	57
60	73·5	70	47·5
70	67	80	36
80	42	90	27
90	20	500	19·5
400	9	10	14
		20	10
		30	6·5
		40	4·5
		50	3
		560	2

8x1

Blank Ans

Emstm 510		Exuli 370	
250	5	400	5
60	7	10	7
70	10·5	20	11
80	13·5	30	12
90	14	40	11
300	13	50	14·5
10	15	60	21
20	19·5	70	29·5
30	28	80	38
40	38	90	45·5
50	44·5	500	50
60	48·5	10	50·5
70	50	20	48·5
80	46·5	30	44
90	39	40	38
400	27	50	32·5
10	15	60	27
20	8	70	21
30	6	560	14
40	4·5		
50	4		
60	4·5		

8x1 rxn

ANS B 5 58 Conc sm as TNS

Quelli (2) ans + Memb

Emstm 6x1 470		Exuli 380·90	
250	6	410	23
60	9·5	20	22·5
70	16	30	29
80	26	40	38·5
90	30	50	48
300	22	60	53·5
10	18	70	56·5
20	19	80	54·5
30	24·5	90	49·5
40	32·5	500	43
50	40	10	36
60	46	20	29·5
70	52·5	30	23
80	57	40	18
90	56	50	14
400	46	60	11
10	30		
20	19		
30	14·5		

6x1 rxn

Li Treated Mamb 6x1

Emm 470		Exuli 380·90	
250	6	410	22
60	9	20	21·5
70	15·5	30	28·5
80	26	40	38·5
90	29·5	50	48·5
300	21·5	60	55
10	17·5	70	58
20	19	80	56
30	24·5	90	51
40	33	500	43·5
50	40	10	36
60	46·5	20	29·5
70	52·5	30	22·5
80	56	40	17·5
90	55	50	13·5
400	47	60	10·5
10	29	70	8
20	17·5	80	5·5
30	13	90	3

(6x1 rxn)

BNS (5x1)
+ Catt trails
nmls

Gain 470		Exhl 380	
250	5	410	13
60	8	20	16
70	15	30	25
80	25	440	37.5
90	28	50	50
300	20	60	58
10	16	70	59
20	18	80	57
30	24	90	51
40	34	500	414
50	42	10	35.5
60	49	20	27.5
70	56.5	30	21.5
80	62.5	40	16
90	60.5	50	12
400	47.5	60	9
10	27.5	70	6.5
20	15	80	4
30	8.5		
40	9.5		

5x1 range

NA trails
nmls

Exmr 470			
250	6.8	410	23
60	10.5		
70	18.5		24
80	29		32
90	32		43
300	22.5		52.5
10			
20	18.5		60
30	20.5		63
40	27		60.5
	36.5		54
	43.5		46
	50.5		38.5
	57.5		31
	62.5		24
	60.5		16
	50		14.5
	31.5		11
	19.5		8.5
430	14.5	560	6

6X1 range

K trails
Gmm 470

250	6	410	23
	10		23
	18		31
	29.5		42
	33		53.5
	23		60.5
	18.5		64
	20		62
	26		55.5
	36		47.5
	44.5		34.5
	52		31.5
	59.5		
	64.5		24.5
	63		19
	51.5		14.5
	32		11.5
	19		8.5
430	14		6

6x1

Cs trails
Exhl 380

250	6		
	10.5	410	24
	17.5		23.5
	29		32
	32		43
	23.5		
	19		53.5
	21		60.5
	27		62
	36		61
	44.5		55
	51.5		47
	58.5		38.5
	64		31
	62		24
	50.5		16.5
	32		14.5
	19		11
	14.5	560	8.5

6x1

15/8/17 ClHCl3 : MeOH 2:1

Thick Opaque Specs
5% TCA
→ Wet pellet CM 2:1 → Centrifuge

Opaque Stains — BSA Dried 1 mg in 1 ml H_2O

1.	H13	0.04	52 / 0.285
2	DND13	0.02	49 / 0.31
3.	LS13	0.04	68 / 0.17
4.	B13	0.04	68 / 0.17
5.	B8,5	DIM2 0.04	80 / 0.0975
6.	B8,5	Dried 0.1	90 / 0.045
7	B8,5	Dried 0.2	84 / 0.075
8.	Blank		100 / 0.0
9	60 µs		85 / 0.055
10	120 µs		81 / 0.0925
11.	180 µs		72.5 / 0.14

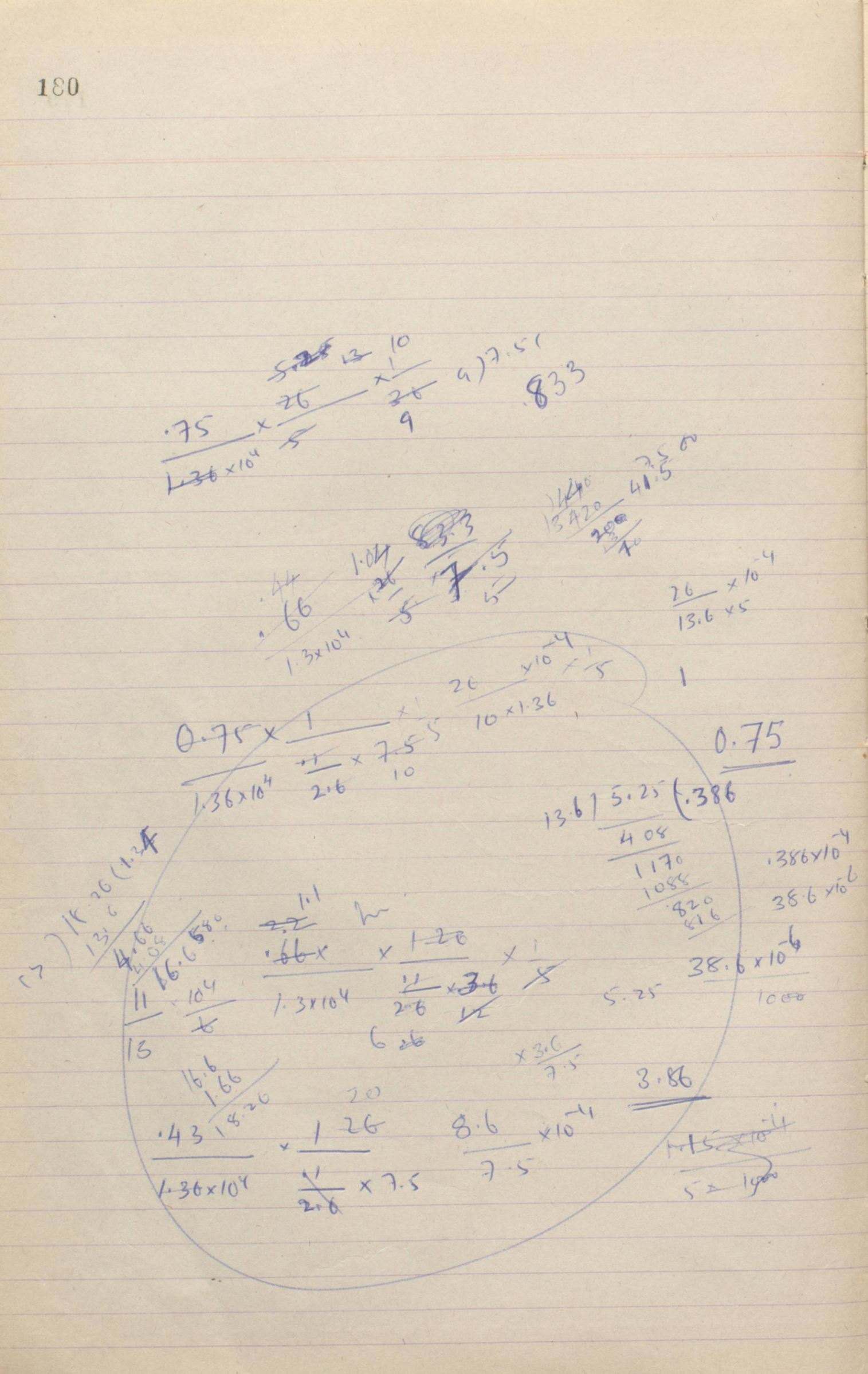
12.04 401.4nm 0.0 15.08 402.4nm

18/8/74 Monkey killed on 16/8/74. Brain kept ~~[illegible]~~
in hrd dry. at 0°C (not frozen) smears made.

(B) 180+25 to 4mm

40.5%

45%

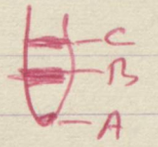


23rd sept-74
23/9/74 Rat brains (Act.D) from MANsanba.

2. Wt 3.2 gms

Homogenate in 6.5% Sucrose | 60 ml. Mitochondria 10:00 - 10:45

Resus 10:45 (H) Centrifuge 3000 rpm 1'
Supt 19000 rpm 10'
Pellet → 3 ml buffer. Kept at 0°.
Layer on 40/28% Sucrose 24000 (64000 g) 30'
Interphase B.



Incubation

A. Cholinesterase 5' incubation 412 nm.
:1 DTNB
:1 ATChCl 2.3 ml
:1 Enzyme + Buffer.

Before substrate addition

Q1	Membrane (B)	0.53/29.5'	1.25'/5.0	3.6 ms/ml
Q2	" (B)	0.57/27	1.35'/4.5'	
Q3	Crude (H)	0.72/29	1.40/3.75	7.5 ms/ml
Q4	" (H)	0.8/11	1.45/3.5	
(+1) Q5	A	0.56/27.5'	1.35/4.5'	
(+2) Q6	C	0.51/31	0.475/10.5	
(B) Q8	NO DTNB	100/0.0	100/0.0	
(B) Q9	No Substrate	0.56/27.5'	0.565'/27.00	
Q10	NO Enzyme	0.35'/44.5'	0.365/43	
~~Q11~~				

300 × 25 / 7.500

24 / 2.5

1.2 × 20 / 7.5

20 / 3.6

ATP .00125 M.

.64 mg/ml.

6
12
16

1.25 mM.

64

.5512 mM. .66 / ml

2 ml 4.62 mg in 6.4 ml.

AMP 3 μM/ml. 10 ml

369 mg/ml : 1 μM

.369 mg/ml : 1 μM 3.69 mg 10 ml

.1 μM.

11 mg / 10 ml 3 μMoles / ml

.23 μM × 25 / 2.5 mg/ml

.1

100

.5

20 / 3.6

23/9/74 Analar
10.45 AM

2+5+0.5

1	Crude H 0.02	74.5 / 0.125	7.5 mg/ml
2	Crude H 0.04	59 / 0.23	
3	Mem B 0.04	75 / 0.12 →	3.6 mg/ml
4	Mem B 0.08	60 / 0.22	
5	Milach A 0.04	66 / 0.18	
6	Myel C 0.08	77 / 0.11	
7	Blank 0.	100 / 0.0	
8	Std. 50 Mg	91 / 0.042	
9	BSA 100	82.5 / 0.085	
10	150	76 / 0.12	

7.40-8.00

Na IC ATPase 30' 540 mμ 5' Nucleotidase 30'

1		45.5 / 0.34	
2	H	45.5 / 0.34	
3	Crude	45 / 0.345	
4	Na	56 / 0.245	
5	B	58 / 0.235	
6	Mem	59 / 0.23	
7		46 / 0.34	
8	H	46 / 0.34	
9	Crude	46.5 / 0.335	
10	K.	56.5 / 0.2475	
11	B	57 / 0.245	
12	Mem	57.5 / 0.24	
13	Blank	100 / 0.0	
14	.1 = 1μm	50 / 0.30	
15	.2 = 2μm	25 / 0.60	
16	.3 = 3μm	14 / 0.85	

.04 mg/ml

1		61.5 / 0.21	
2	H	63 / 0.20	
3	Crude	61.5 / 0.21	
4		71 / 0.15	
5	B	71 / 0.15	
6	Mem	71 / 0.15	
7	Blank	100 / 0.0	
8	1μm	50.5 / .2975	
9	2μm	26 / 0.585	
10	3μm	14 / 0.85	

0.03 mg/ml

Ach 250mM 429.25/10ml

751/4 = Gly · 250mM 186 mg/10ml

ATP

3)9.62 ×2

1.54 ×2 : 3.08 mg/12 5min 4.8 ml

Can prewarm to 16min

AMP

8.8 mg/8 5min

Autosampler 10 hrs

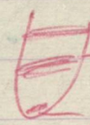
B 239

{2 3 sept prep
→ catalysed
Freeze thawed}

24 sept 74
24/9/74

2 Rats - Male Brain wt 3:00 pm

60 mg 8.5 sucrose in pH 7.4 10mM hepes/Tris.
Homogenate (H) 24/9.
Spun 3500 1' pellet DNA.
Supt spun 10,000 10' pellet.
Resuspended in 30 ml hepes.
Layered over 40 25% sucrose Myelin B 249.
C 249 & A 249 also collected.



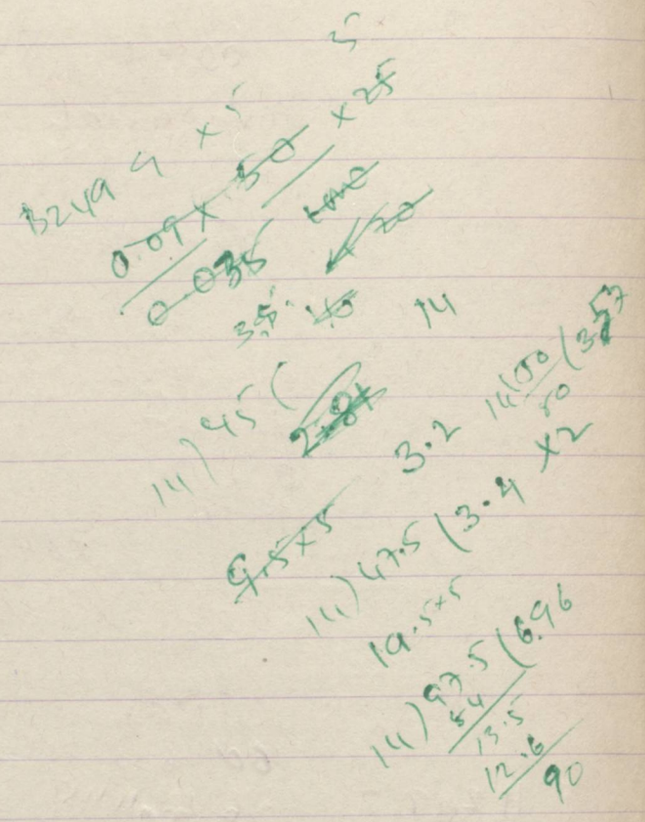
Total 9 ml B (made up) (9 ml)
8 ml for dialysis. 540 mμ

Na-K ATPase

		60' 655-725	
1	H 249	36	0.445
2	H 249	35	0.455
3	B 249	51.5	0.29
4	B 249	52.5	0.28
5	H 239	39.5	0.405
6	B 239 D	67	0.175
7	H 249	39	0.405
8	H 249	36.5	0.435
9	B 249	52.5	0.28
10	B 249	54	0.27
11	H 239	41.5	0.38
12	B 239 D	59	0.23
13	Blank	100	0.0
14	1 μM	48.5	0.315
15	2 μM	24	0.615
16	3 μM	12.5	0.9

5' nucleotidase

		30' 655-725	
17	H 249	70.5	0.15
18	H 244	72	0.1425
19	B 249	81	0.09
20	B 249	80.5	0.095
21	H 239	71	0.15
22	B 239 D	88	0.05
23	Blank	100	0.0
24	1 μM	50.5	0.30 (.295)
25	2 μM	27	0.57
26	3 μM	13.75	.863



2419 pH 4 protein 540mμ (2 + 0.5 + 5) ml

	ml		
B 249 (Mrs Indrajha)	.04	81.5 / 0.09	3.2 mg/ml
B 249	.08	67 / 0.175	
H 249	.02	80.5 / 0.095	6.8 mg/ml
H 249	.04	64 / 0.195	6.96 mg/ml
B 239 D (Dilated 23/9/54 Mrs)	.08	79.5 / 0.1	1.78 mg/ml
Blank		100 / 0.0	
BSA 50 μg		92.5 / .035	
BSA 100 μg		83.5 / 0.0775	
BSA 150 μg		77.5 / 0.115	
BSA 200 μg		70 / 0.155	

Free Phosphatase mixture — No blue colour No Turbidity

Mems	77 / 0.115	1 ml sample + 0.3 ml TCA (30%) + 1.5 (molybdate) + 0.2 (MMSA) + 8 ml water.
Cmale	83 / 0.0825	
Blank	90 / 0.045	

3 mg/ml

Too Complex mix

(B.C.W)

Δ^{239} D Diabetes
8 Weeks Natural
Newborn 9 Mos

Δ^{239}D ⇈
⇊

I Free
Na
ACh
GABA

II GLY
K
CHOL
Ca

25/9/14 NO DTNB = 100% 412 nm

A. cholinesterase (0.1 + 0.1 + 0.1 + 2.3 buffer)

	0	30	60	90	120	150	180
1 H 249	29 .538	24.5	20.5	18	15.5	13	11.5 .440
2 B 249	42.5 .372	36	30.5	26	22	19	16 .796
3 H 239	23 .638	18	14	11.25	9	7.5	6.5 1.186
4 B 239 D	46 .337	40.5	36	32	28	25	22 .654
5 H 249	29 .538	24.125	20.5	17.5	15	13	11 .959
6 B 249	43 .367	35.5	30	25	21	18	15 .824
7 B 239	35.5 .450	28	22.5	18.5	15	12	10 1.000
8 NO Enzyme	61	—	60.5	—	60	—	
9 NO DTNB	100						
10 NO Substrate	56.5	—	56	—	55.5	—	

I set- (Mems 24/9 DIAL / Mems addition) TNS : 0.2 mg/ml Solution

4 wells: 8.4 ml buffer + 1.2 ml TNS = 9.6 ⟹ 2.4 each to I, II, III, IV + 0.6 buffer / + 0.6 Substrate / "

Addition: 2 ml Mems + 0.4 ml TNS } .6 ml to I, II, III + 0.4 ml buffer / + 0.2 ml buffer + 0.2 ml Substrate

II set- (8.4 ml buffer + 1.2 ml Mems) ⟹ 2.4 ml + I + 0.6 ml buffer / II + 0.6 ml Substrate

Addition- 2 ml TNS + 1.4 ml Mems → .6 ml each → Add I + 0.4 ml buffer + 0.2 ml buffer + 0.2 ml Substrate

130 1st set

TNS : 57 μMoles in Mems added Conc

Mems 1.805 mg/ml ⇒ 0.9025 conc/ml in reciprocals

Mems = 180.5 mg/ml in TNS added Conc

TNS 285 μMoles/ml in reciprocals

Dlo	Dlo.
1/51 T. 2924	9/59 T. 1833
2/52 T. 5850	10/60 T. 2218
3/53 T. 7528	11/61 T. 2561
4/54 T. 8694	12/62 T. 2868
5/55 T. 9586	13/63 T. 3146
6/56 T. 0300	14/64 T. 3399
7/57 T. 0892	15/65 T. 3632
8/58 T. 1392	16/66 T. 3846

(Conc.)

.3 ml TNS + .3 ml Mems (0.7 × 0.5 ml · (mg/ml)$^{-1}$)

+ Mems added (mg/ml) ⇒ Reciprocal

1	56.53
2	28.82
3	19.59
4	14.97
5	12.19
6	10.34
7	9.001
9	7.266
11	6.140
13	5.370
15	4.801

Reciprocals

25/9/74 360 430

range	I Free	II Na	III ACh	IV CHAMBA.	Membr Addition (Sum)
4×1	1	1	1	1	0.0
	10	12	13	9	0.05
	19.5	22.5	28	16.5	0.05
	29	34	42.5	24.5	0.05
	38	43.5	57	32	0.05
	46	53	71	38.5	0.05
	56 (4×1)	60.5 (4×1) / 33 (3×1)	87.5 (4×1) / 47 (3×1)	45 (4×1)	0.05
	62.5 (4×1)	70.5 (4×1)	105 (4×1) / 60.5 (3×1)	53 (4×1)	0.05
	76 (4×1)	85 (4×1) / 45.5 (3×1)	111 / 76 (3×1) / 146	62 (4×1)	0.1
	88 (4×1) / 47.5 (3×1)	97 (4×1) / 53 (3×1) / 97	— / 95 (3×1) / 183	74 (4×1) / 40 (3×1)	0.1
3×1	100 (4×1) / 54 (3×1)	— / 60 (3×1) / 113	100 (3×1) / 67 (2×1) / 251	85.5 (4×1) / 46 (3×1)	0.1
	62 (3×1)	69 (3×1) / 131	69 (2×1) / 261	53.5 (3×1) / 101	0.1

← Scale adjusted.

TNS added	Reciprocal
	0.1789
	0.09119
	0.0620
	0.04734
	0.03861
	0.03275
	0.02862
	0.02301
	0.01940
	0.01701
	0.01520

0.5 ml mixture + .75 x .5 ml TNS (1/2 in 1st set reading 5)

(rechecked)

360-430

4x1 mg.	I Free	II NA	III ACh	IV GABA	TWS selection RS
	4	3.5	4.5	4	0.0
	17	21.5	22.5	16	0.05
	27.5	36.5	39	25	0.05
	37	49	51.5	35	0.05
	46	62	64.5	41.5	0.05
	53	70	72.5	46	0.05
	60	78	79.5	51	0.05
	66	(87) 87.0 (4x1) } 89	89 (4x1) }	59 (4x1) }	0.05
	—	(47) 47.0 (3x1) }	48 (3x1) }	— }	
	73.5 (4x1)	97 (4x1)	96.5 (4x1)	64 (4x1) }	0.1
	—	52 (3x1)	52 (3x1)	34.5 (3x1) }	
	82 (4x1)	over 100 ~~104 (4x1)~~	102 100 (4x1)	69.5 (4x1) }	0.1
	44.5 (3x1)	57 (3x1) (10)	54.5 (3x1)	38 (3x1) }	
87	47 (3x1)	60 (3x1) 113	111 59 (3x1) 113	74 40.5 (3x1)	0.1
103	54 (3x1)	65 (3x1) 123	60 (3x1)	79 43 (3x1)	0.1

TNS added

Reciprocal.

1 0.1789

2 0.09119

3 0.0620

4 0.04739

5 0.03861

6 0.03275

8 0.02548

10 ~~0.02301~~

12 0.02106

14 0.01813

16 0.01604

0.01447

* Initial Adjustment

360 - 430

9 .. 16 Free, Ach, Gly, Chd 4x1

(TNS ADDED Gly)

9 Free	10 Ach.	11 Gly	12 Chd.	Gly
	3	3	3	0.0
3				
22*	16	14.5	17	0.05
35	29	24	28.5	0.05
44	40.5	33.5	41.5	0.05
54	54	41	53.5	0.05
62.5	67	48	59	0.05
70	76	55	67	0.05
			78	0.1

80 (4x1) 87 (4x1) 63.5 —

83.5 (3x1) (4x) 47.00 (3x1)

91 (4x1) overlook (4x1) 72 (4x1) 90 (4x1) 0.1

56 (3x1)

97 (4x1) overlook 79 (4x1) 92 (4x1) 0.1

60 (3x1)

3x1

57 107 67 40 48.5 100 53.5 0.1

58 109 68.5 54 48 101 54 0.1

mg/ml

Mbrs added — Neuprocat.

1	56.53
2	
3	28.82
4	19.59
5	
6	14.97
7	
8	12.19
9	
10	10.34
11	
12	
13	
14	
15	

9.001

7.266

6.140

5.370

4.801

360-430

Free	Ach	Alt	Clutch	Membadded
13	14	15	16	Alt
1.5	1.5	1.5	2	0.0
10	16	10	11	0.05
18	29.5	17.5	20.5	0.05
25.5	42	25.5	29	0.05
33	55	32.5	37.5	0.05
40	68	39	45	0.05
47	83 (4x1) / 45 (3x1)	46	54	0.05
53	96 (4x1) / 52 (3x1)	52	60	0.05
62.5 (4x1)	126 66.5 (3x1)	62	72.5	0.1
74	144 80.5 (3x1)	72.5	84 (4x1) / 45 (3x1)	0.1
83.5 (4x1) / 45 (3x1)	180 93.5 (3x1)	83.5 (4x1) / 45 (3x1)	95.5 (4x1) / 51 (3x1)	0.1
3x1 91.5 49	220 57 (2x1) / 100 (3x1)	49.5 (3x1)	165 55.5 (3x1)	0.1

B 249D

.065 × 25 × 50 / 1000 = 4 / 20 = 1.805 avg/mile

B249D 9)65 (7.22 = 2.64
4
B239 9)95 (10.55
4
B235 9)115 (12.77 = 3.19
4
B226 9)150 (16.66 = 4.14
12
9)125 (13.9 = 1.74
9

25/9/74
Prolein

	ml		
Mmb B 249 D (Dialysed)	.04	86 / 0.065	(1.805 mg/ml)
Mmb B 249 D (Dialysed)	.08	74 / 0.13	
" B 239 D (Dialysed fresh Mamea)	.04	80.5 / 0.095	2.64 mg/ml
" B 234 D	.08	75 / 0.125	or 1.74 mg/ml
Mmb B 249	.04	77 / 0.115	3.19 mg/ml
Mmb B 234	.04	71 / 0.15	4.14 mg/ml
Blank		100 / 0.0	
50 µg		90.5 / 0.045	
100 µg		82 / 0.09	
150 µg		73 / 0.135	

20/9/74

A cholinesterase in CISL U.V. Spectrophotometer recording. Instruction

27/9/74

25/9/74 Binding Analysis

1 - .. 16 tubes TNS.

+ after Centrifugation 15' 8000 rpm.

Supt's.

17 Blank

18 .1 ml TNS (.2mg/ml) with 2.9 ml supt. (Total 3ml in cuvette)

19 .2 " " 2.8

20 .3 " " 2.7 "

21 .4 " " 2.6 " "

Feeding this 20

Blank 100 %
(only -0- in Xylene
(Blank to 100
at any
wavelength).

300	26		
305	14	307	11.5
310	9	309	10
315	7.5	311	8.5
		313	8.
320	10	3111	7.5
325	17	316	7.5 - 8
330	21		
335	33		
340			
345			
350			
355			
360			
365			
370			
375			

315 nm

315 ready

1 7 - 6.5

2 8 - 8.5

3 15

4 5 - 5.5

5 5.

6 6

7 9.5 - 10

8 3 - 3.5

9 5.5 - 6.

10 10 - 10.5

11 4

*12 3 - 3.5 (outra fita karenka)

13 5 - 5-5

14 13.5 - 14

15 5 - 5-5

16 9 -

17 100

18 32.5

19 13

20 7 - 7.5

21 4 - 4.5

23/9/74

0.5 ml TTX (.1 mg/ml)
injected to 20 gm weight rat (15 days old)
45 min second convulsion & death in 1 ml -
1 - 2 ml writhing & Jaw.
Shiny chocolate (glassy) brain 1.05 gms. / rat (3 gms)

(Adult rats females) 2. 2.7 gm brain.
NO 2 hr sep. white ppt. & clearing → poor separation.

Crude HN 27	BN	12 ml	Gelly
Crude HT 27	BT	4 ml	

(5 ml B239 DIAL + 5 ml ANS + 25 ml buffer.
2 ml in + 0.5 ml sol- in buffer. & freeze - Act D treated muscle
Enzymes.

Free. Exalut 390 3 x 1 range. ??

	Free	Na	K	Li	Cs	Ach	Chol	GABA	GLY
420	12	14	13	13	15	14.5	15.5	12.5	neat
430	17	20	20	20.5	23	21	23.5	17.5	Ora
440	25	30.5	30	31.5	34.5	31	35	24.5	
450	35.5	44	41.5	45	51	44.5	47.5	33.5	
460	40	52	48	53	61.5	52	56	39	
470	43	57	51.5	57	60	55	60	41	
480	41.5	55	50	54.5	58	53.5	57	38.5	
490	37	48	44	47.5	51	47	49.5	33.5	
500	31.5	40	37	40	43	39.5	42	28.5	
510	25.5	32.5	30	33	34.5	32	34	23.5	
520	20	25	23	25.5	27	25	26	18	
530	15	18.5	17	19	19.5	18	19	13.5	
540	11	13	12.5	14	14.5	14	14.5	10.5	
550					11	10.5	10.5	8	

Gdn 0.25M (partially filled) Impl = .2M

MA .05M

Tree (Last ready)

3×1

14
21
29
40
47
50
47
41
35
27.5
22
16.5
12.5
9.5

3x1 rays

	GLY	Glu*	NA	Ca++
420	15	52	42	21
	12.5 (2x1) / 23 (3x1)	64.5 (2x1)	96.5 (3x1) / 57.5 (2x1)	33.5 (3x1)
	34 (3x1) / 18 (2x1)	(1x1) 64	101 (2x1) / 51 (1x1)	30.5 (2x1) 63
	21 (2x1)	96 (1x1) / 39 (3x1/10)	80 (1x1) / ~~34~~ 33.5 (3x1/10)	40 (2x1) 82
	25 (2x1)	46 (3x1/10)	40.5 (3x1/10)	43 (2x1) 88
(53)	27.5 (2x1)	50.5 (3x1/10)	48.0 (3x1/10) (470)	51 (2x1) 103
	27 (2x1)	50 (3x1/10)	47 (3x1/10)	49 (2x1) 100
	23 (2x1)	46 (3x1/10)	43 (3x1/10)	44 (2x1) 90
	19.5 (2x1)	38.5 (3x1/10)	37 (3x1/10)	36 (2x1) 77
	15 (2x1)	31 (3x1/10)	30 (3x1/10)	28 (2x1) 58
	12.5 (2x1)	25 (3x1/10)	25 (3x1/10)	22 (2x1) 46
	9 (2x1)	74 (2x1)	75 (2x1)	16.5 (2x1) 35
	6.5 (2x1	50 (2x1)	52.5 (2x1)	11 (2x1) 24
350	5 (2x1)	36 (2x1)	36 (2x1)	8 (2x1) 18 / 550

Free: 2x1

Cal. reads < 420

7.5 (2x1)
11 (2x1)
17 (2x1)
23 (2x1)
26 (2x1)
27.5 (2x1) 470
26 (2x1)
23 (2x1)
20 (2x1)
15 (2x1)
12
9
6.5
5 (550)

ATP
5.77 mg / 20 kcal / 1 ml (?)
→ 3.2 ml
(1.5)

Amp 11 mg / 10 cm³

27/9/74 — 45' — 30'

Na⁺ K⁺ ATPase — 5' nucleotides

.04 ml 7-43-828 — 743.813

1	HN27	32%	.495	
2	HN27	32%	.495	
3	HT27	56.5%	.248	
4	HT27	55%	.260	
5	BN27	36%	0.444	
6	BN27	36.5%	0.438	
7	BT27	73.5%	0.134	
8	BT27	70%	0.155	
9	HN27	31	0.509	
10	HN27	31.5	0.502	
11	HT27	48	0.319	
12	HT27	46	0.337	
K⁺	BN27	34	0.469	
14	BN27	34	0.469	
15	BT27	61	0.215	
16	BT27	62	0.208	
17	Blank	100	0.0	
18	1 μM	50.5	0.296	
19	2 μM	25.5	0.593	
20	3 μM	13.5	0.870	

[0.7+0.04+0.3+1.5+0.2+5.7]

.05 ml

21	HN27	68	0.168
22	HN27	67	0.174
23	HT27	80	0.097
24	BN27	70.5	0.152
25	BN27	63.5	0.197
26	BT27	91	0.041
27	Blank	100	0.0
28	1 μM	48.5	0.314
29	2 μM	26.5	0.571
30	3 μM	13.5	0.870

[1.2+0.03+2.5+0.2+37]

Grade 100 x 50 =
1 IN 2?

HT 3.75

40

x 25
115

3.83

115 x 25 x 100

075 2

15 25/30 24

BT: 03

35/30 = 1.166

27/4/24 Protein estimation

540 mμ.

1	HN27 .02	84 / 0.075	5 mg/ml
2	HN27 .04	68.5 / 0.155	
3	HT27 .02	88 / 0.055	3.75 mg/ml
4	HT27 .04	76 / 0.12	
5	BN27 .04 (.04)	77 / 0.115	3.83 mg/ml
6	BN27 .08 (.08)	59 / 0.23	
7	BT27 .04 (.04)	92.5 / 0.035	1.166 mg/ml
8	BT27 .08 (.08)	85.2 / 0.07	
9	Blank	100 / 0.0	
10	50 μg	90.5 / 0.045	
11	100 μg	84 / 0.075	
12	150 μg	76 / 0.12	
13	200 μg	70.5 / 0.15	

(N + 64 0.4)

483.23
+
460
23.23

5 dosis mg

21 x 23
101
27 x 29.41

483.27
794.07

5.40
245
10.8
.127
794.07

127.7

127
121.3
45
14 4

1,45 mg

,173
.14
,1277

1 ml 1.277 mg.

1.14 ml = 1.45 mg

28/9/74

Citrate buffer

A: 0.1M citric acid 21.015 g/litre

B: 0.1M Sod citrate 29.41 g/litre

(as the system)

x ml A : 23 - x dilute with H_2O 1:1

y ml B : 27 - y Incell & Somme

$\Rightarrow$ 483.23 mg citric acid } ratio
794.07 mg sod citrate }

48.3 mg
79.4 mg } 127.7 mg.

TTX 4.60449
4.60478
.00029 0.29 mg

TTX I · 29 mg in 1.14 ml citrate (127.7 g/100 ml) + 4.86 ml H_2O
in 2 ml
Iodine

TTX I / 10 to fold dilute TTX I / 100 = TTX I / 1000 ec

$\frac{6}{7}$

I : Free

II $\frac{1}{10000}$

III $\frac{1}{1000}$

IV $\frac{1}{100}$

V $\frac{1}{10}$

VI $\frac{1}{}$

BN27

Range [1x1] 365 - 425 Maxima. 213

Wee	Ach	K	Na	Qq
38	38	36.5	38	0.0
38	39.5	40 1.5	40.5	0.05
exact? 39	41.5	42	43	0.05
(1x1) 38.5 / (2x1) 74.5	42.5 / 80	43 / 81	44 / 83	0.05
2x1 74 36.5	83 41	84.5 45	87 46.5	.05
74 36.5	86 43	87 46.5	89 47.5	0.05
74 36.5	91 46	90 48	89 47.5	0.05
74 36.5	94 (2x1) / 50 (1x1)	90.5 / 48.25	92.5 / 49	0.1
74.5 36.5	98 (2x1) / 52 (1x1)	93.5 (2x1) / 50 (1x1)	95.5 (2x1) / 51 (1x1)	0.1
74 36.5	over 100 / 58 (1x1)	95.5 (2x1) / 51 (1x1)	98.5 / 52	0.1
74 (2x1) / 39.5 (1x1)	over 100 / 56.5 (1x1)	57.5 (1x1)	53 (1x1)	0.1

(Ach perhaps Phelps)

II

2x1 rxn

Free	Ach	K	Na	Q4
42 80	81	79.5 42	79 41.5	0.0
42 90 (79.5)	81.5	83.5 43.5	80.5 42 *	0.05
42 79.5	84.5	85 44.5	84 44	0.05
42 79.5	89.5	87.5 43.5	86.25 45	0.05
42.5 81.5	96 } 51.5 }	90 49 47	87 (2x1) } 45.5 (1x1) }	0.05
42 79.5	over 100 } 56 }	92 (2x1) } 49 }	90 (2x1) } 48 (1x1) }	0.05
79.5 (2x1) } 42 (1x1) }	59.57 (1x1)	49.5	48.5	0.05
(1x1) 42	66.5	51	50.5	0.1
42	70	51	51	0.1
41	73	52	52	0.1
42	74.5	52.5	52.5	0.1

*

III	Free	Ach	K	Na	Ag
2x1	44 83	82.5	80.5 43.5	80.5 43.5	0.0
43	~~82.5~~ 81.5	82.5	82.5 44.5	83 44.5	0.05
43	81.5	86	83.5 45	86 46	0.05
43	81.5	90	86 46	90 44	0.05
43	81.5	(2x1) 94 (1x1) 50	90 48	90 48.5	0.05
	81.5 (2x1) 43 (1x1)	97.5 53	90.5 49.5	90.5 48.5	0.05
(1x1)	42.5	55	48.5	47	0.05
↓	42	57.5	48	48.5	0.1
	42.5	61.5	49.5	50.5	0.1
	42	63	50	52	0.1
	41.5	64.5	50	51.5	0.1

Kept in cold 4 2 hrs

IV 2 x 1 rye

Tree	Ach	K	Na	Ca
72 39	70	69 38	70 38.5	0.0
73.5 40	82.5	77 41	76.5 42	0.1
72 39	94.5 2x1 50 1x1	80.5 43.5	82.5 44	0.1
72 39 (1x1)	101 54	86 46.5	90.5 48.5	0.1
(1x1) 39 (1x1)	58	48	50	0.1
40	60.5	49.5	57.5	0.1
40	63.5	50.5	53	0.1
40	65.5	51	53.5	0.1

V

	Fe	Ach	K	Na	Ni
1x1	79 42.5	83 44.5	81.5 43.5	82.5 44	0.0
	80 43	87 46.5	87.5 46.5	88.5 47	0.1
	80 43	89.5 47.5	90.5 48	91.5 49	0.1
	79 42.5	91 48.5	92 49	92.5 49.5	0.1
	78	95 (2x1)	93 (2x1)	92 (2x1)	0.1
	42	50.5 (1x1)	49.5 (1x1)	49. (1x1)	
	42	53	51.5	51	0.1
	42	55.5	52.5	52.0	0.1
	42	58	52.5	53	0.1

VI

	Free	Arch	Ic	Na	Ag
2×1 rx	76 41	76 41	75 40.5	75.5 40.5	0.0
	77 41.5	78.5 42	80.5 43	81 43.0	0.1
	76 41	79 42.5	83.5 44.5	83 44.0	0.1
	75.5 41	82 44	85.5 45.5	86 45.5	0.1
	77 41.5	86.5 46	87.5 46.5	90 47.5	0.1
	(no more Arch)				
74	40	86 *	87	46.5 89.5	47 0.1
74	40	86.5 *	90.5	48.5 93.5	49 0.1
74.5	40	88.5 * (92)	93	49.5 94.5	50.5 0.1

BN27 Spectia

1 ml BN27

1.5 ml ANS (not TNS)

7.5 ml Buffer.

2.5 ml + 5 ml Sall. a buffer h speed.

Tree	A.ch.	Ch.cl?	CVa (2 x 1 yr)
8	8.5	9	8.5 420
11.5	12	12.5	12.5
17.5	18	19.5	19.5
23.5	25	26	27.0
28	30	31.5	32.5
30	32.5	34	35
29	32	33	34
26	28	29	30
22	24.5	25	26
17.5	19.5	20	21
14	15.5	15.5	16
10.5	11.5 (12)	12	12.5

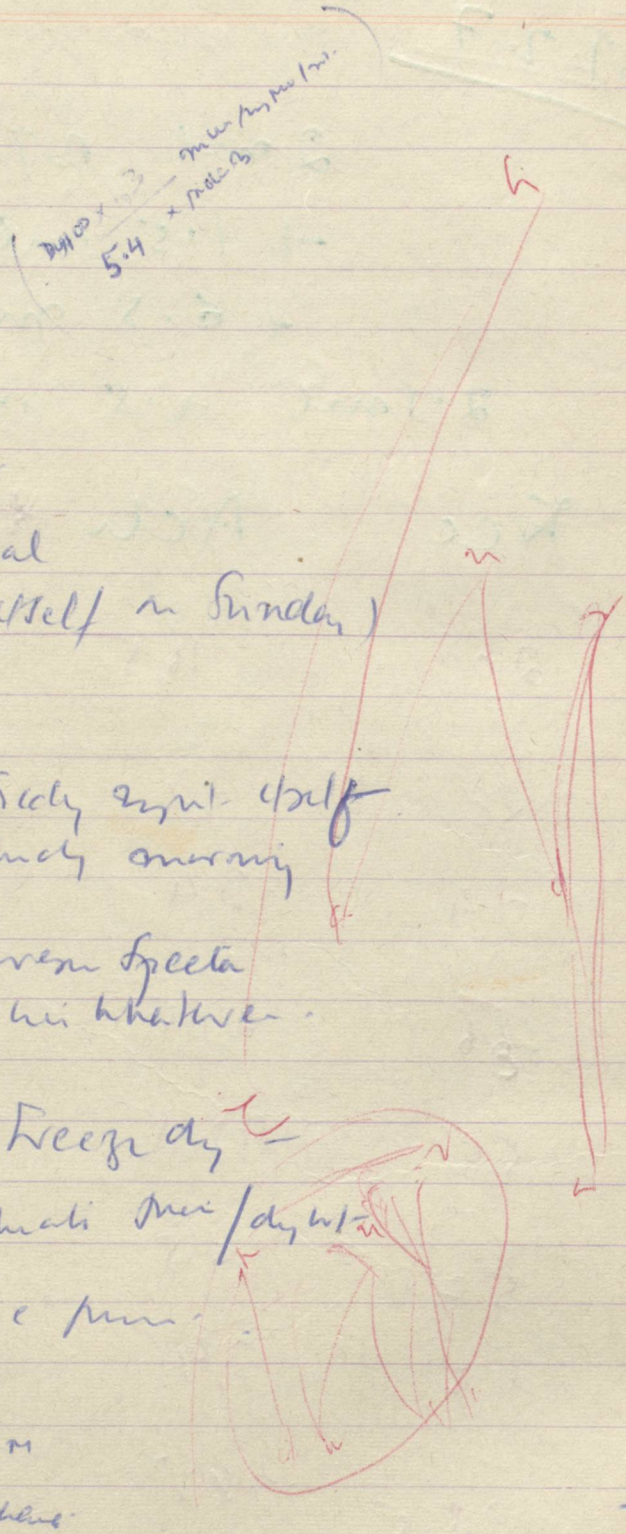
TNS 365 - 425

(Hl dulcicd [illegible] Samaro
[illegible]
(blanc dulcis
Lin Acr.)

BT27

2 ml BT27
+ 1.5 ml TNS.
+ 6.5 ml buffer
2.5 ml + 5 ml salt or sugar (2 x 1 rage)

Free	Ach*	Na+	K+	
				390
20	16.5	22	22	400
28	23	33	32	
44	34	53	54	410
~~66~~	41.5	66	67	420
56	42.5	~~69~~	71	430
52	39.5	65	66	440
42	34	56	54	450
38.5	28.5	46	46.5	460
29.5	24	37.5	38	470
22.5	18	29.5	30	480
16.5	14	21	~~16.5~~ 21.5	490



5.4

(Old preparation
Nolen Manual
In Schultz Hilf u Sunday)

New preparation
Enzym Fraktion zugibt Hilf
& Salzsäure anwenden

Salzsäure Thymen Spezial
hinzufügen.

Lipp aus Kreuzdrüse
Eiweiß Menge / drüse

Leucin ...

EDTA .312 mM

100 bei dem

31.2 mM →

31.2 x .37225 x

3,1223 x 31.2

7446
3723
1169
116.1576

(SDH Gut Lactat etc für Stoffwechsel)

30/9/74 A. cholinesterase enzyme related in
HN27 HT27 BN27 [illegible] 27.
AMO reached TMS fig 4 12 ... 16.
[illegible] brain [illegible]

SDH [illegible] ms:

	0	15	30	45	60	
HN27	35	36	37	38	39.	0.1 ml
HT27	40	41	41.5	42.5	43	0.1 ml
BN27	34.5	35.5	36	37	38.5	0.1 ml

1/10/74 [illegible]

3/10/74 Solution for 4/10/74. Was prepared
5 incubation
① Gly - NaOH PH 8.5 1.0 M. Ref. [illegible]

Na K ATPase

A NaCl 0.25 M $MgSO_4$ 0.00138 M

B A + 0.0125 M KCl.

D. Tris Hcl 0.0625 M (PH 8.6
NaEDTA 3.12×10^{-4} M)

NADH Diaphorase

A: 0.02 M Tris Hcl PH 7.4
B 0.003 M Pot Ferricyanide
C. DPNH 2 mg in 10 ml

.4 ml A
.4 ml C
.2 ml B
(40-20 µg protein)

340

In Enzymology

Tris-HCl buffers

A 0.2M Soln Tris 24.2gm in 1000ml

B 0.2M HCl

50ml A xml B Dilute to 100ml (w/ dH2O)

x	pH
5.0	9.0
8.1	8.8
12.2	8.6
16.5	8.4
21.9	8.2
26.8	8.0
32.5	7.8
38.4	7.6
41.4	7.4
44.2	7.2

Thursday 3 months - Take

Buffer Solns ① Gly-NaOH 1M PH 8.5 (100 ml)

Mix At 37°C → ② Tris Hcl PH 8.6 0.1M. (100 ml)

PH at ~~25~~°C ③ Tris Hcl PH 7.4 1M. (50 ml)
~~(make 7.0)~~ (7.2)

Others ① 5 mm 6% in 10mM PH 7.4 Sm/p.

② pot Ferrocyanide 0.003M

③ Get DPNH. 10 mg in 50 (2 mg in 10 ml)

④ NaCl 1.25 M $MgSO_4$ .0013M

⑤ ④ + KCl .0125 M

---

① Gly-NaOH

7.505 gm in 50 ml (2M) Gly
~~76~~ 8 ml 1M NaOH
Dilute to 100 ml. } Check PH before making up

② ~~2 M~~ 1.21 gm Tris in 50 ml } Check PH & make up to 100 ml
in Tris Hcl & add
+ 12.2 ml 0.2M Hcl

③ Tris ~~2 M~~. 60.5 gm in 250 ml. } Check PH
+ 2M Hcl 220 ml
Make up to 500 ml

Gly NaOH (.05 M)

A : 0.2 M soln Gly (15.01 in 1000 ml

B : 0.2 M soln NaOH

50 ml A + 21 ml B
dilute to 200 ml

ml	pH
4.0	8.6
6.0	8.8
8.8	9.0
12.0	9.2
16.8	9.4
22.4	9.6
27.2	9.8
32.0	10.0
38.6	10.4
45.5	10.6

4/10/74 1 Adult male → Motor Cortical region

Numbers 2 Young male brains kept

3 Young female → Motor Cortical region

II Female 2.4 gm. | 25 ml/gm homogenising mixture 5% sucrose in 2.4 TM

I Male 3.3 gm

III Rat 2.8 gm

I H^M Male Motor Cortical homogenate

II H^F female " "

III H^R Rat " " (freeze thawed)

Final Collection I B^M – Q 17 10 ml

II B^F – Q 18 10 ml

III B^R – Q 19 5 ml.

2½ hrs time 10,00 hrs Evening Morgue

$.02 M \pi \times 1$

8 1 mi

(2 4 6 9 Blue Color Sphere Beads)

.05

9.10.74

451

8-57 – 9-42 PM 540 m/h

.05 mil much

Na

K

1	H I M	31.5 / 0.50	
2	H I M	30 / 0.52	
3	H II F	36 / 0.44	
4	H II F	35.5 / 0.45	(Smoking Wrong)
5	H III R	36 / 0.44	
6	H III R	32 / 0.49	
7	B I M	46 / 0.32	
8	B I M	48 / 0.32	
9	B II F	46.5 / 0.335	
10	B II F	47 / 0.33	
11	B III R	57 / 0.245	
12	B III R	57 / 0.245	
13	B I TMT	45.5 / .34	
		45.5 / 0.34	
14	B II ~~F~~ T	55 / 0.26	
15	B III RT		
16	H I M	31.5 / 0.58	
17	H I M	35 / 0.46	
18	H II F	36 / 0.44	
19	H II F	38 / 0.42	
20	H III R	36 / 0.44	
21	H III R	40 / 0.395	
22	B I M	48 / 0.32	
23	B I M	49 / 0.31	
24	B II F	47.5 / 0.325	
25	B II F	46.5 / 0.33	
26	B III R	53.5 / .275	
27	B III R	55 / 0.26	
28	B I MT	47 / 0.325	
29	B II FT	48.5 / 0.315	
30	B III RT	56.5 / 0.245	
31	Blank	100 / 0.0	
32	1 MM	– 47.5 / 0.325	
33	2 MM	– 24.5 / 0.61	
34	3 MM	– 14 / 0.85	

	Protein	5' nucleotidase
H I M	9.375 mg/ml	2 μMol/mg/hr
H II F	6.25 mg/ml	2.13 μMol/mg/hr
H III R	6.87 mg/ml	1.75 μMol/mg/hr
B I M	2.8 mg/ml	3.81 μMol/mg/hr
B II F	2.65 mg/ml	6.1 μMol/mg/hr
B III R	1.1 mg/ml	6 μMol/mg/hr

6.1

6.0

3.8

6.21

.085

2.

1.75

9:00 - 9.30 PM

5% Nucleohas .05 — 30' 540 mμ — 540 mμ — Protein 2 + 5 + 0.5

1	H I M	71 / 0.15	1	H I M .02	70 / 0.155
2	H I M	72.5 / 0.14	2	H I M .04	57 / 0.295
3	H II F	79.5 / 0.1	3	H II F .02	76.5 / 0.115
4	H II F	80.5 / 0.095	4	H II F .04	64.5 / 0.19
5	H III R	82 / 0.0875	5	H III R .02	72.5 / 0.11
6	H III R	83 / 0.08	6	H III R .04	
7	B I M	83 / 0.08	7	B I M .04	60 / 0.22
8	B I M	63 / 0.082	8	B I M .05	80 / 0.0975
9	B II F	76 / 0.12	9	B II F .04	65.5 / 0.145
10	B II F	75 / 0.125	10	B II F .05	78 / 0.105
11	B III R	87.5 / 0.06	11	B III R .04	67 / 0.17
12	B III R	91 / 0.04	12	B III R .05	
			13	Blank	100 / 0.0
			14	50	93.5 / 0.03
			15	100	53 / 0.08
			16	150	100 / 0.0
			17	200	91 / 0.0425
					83 / 0.0825
					75.5 / 0.125
					69 / 0.165

Blank 100 / 0.0

1 μM 49 / 0.31

2 μM 27 / 0.57

3 μM 14 / 0.85

5/10/74 Re Run in Gangoly & Lagnes Ruff.

4.128 Mercis I M II = III R

B' I M B' II F B' III R

B (I + II) D B I M A II F

Dwight -

Protein

B' I M 3.33 mg /ml . 5.7 mg ATD

3 ml

B' II F 2.7 mg /ml

30 x .4

12 ml

B' III R 2.08 mg /ml

12 ml

B (I + II) D 2.77 mg /ml

.4 ml

.16 .

$\frac{.12}{.045} \times \frac{25}{9} \times \frac{50}{1000}$

$\frac{10}{3}$ — 3.33 — $\frac{11}{4}$ — 2.77

$\frac{10}{3}$ 3.33

$\frac{.075}{.045} \times \frac{5}{9} \times \frac{50}{4}$ $.25 \times 25$ $\frac{625}{3}$ 2

0.3 3

$\frac{.025}{0.3} \times 25 \times \frac{4}{3}$ $\frac{2.5}{0.9} \times \frac{1}{9.375}$ $\frac{0.6}{0.9 \times 4.375}$ $\frac{2.5}{6.37}$ 0.3

$\frac{.035}{0.3} \times 25 \times \frac{4}{3} \times \frac{1}{6.25}$ $\frac{3.5}{0.9 \times 6.25}$ $\frac{7}{11}$ .636 $\frac{1}{11}$ .09

.01 $\frac{1}{0.9 \times 6.87}$ $\frac{1}{6.1}$ 0.16

$\frac{.025}{0.3} \times 25 \times \frac{11}{3} \times \frac{1}{3.8}$ $\frac{2.5}{0.9 \times 3.4}$ $\frac{2.1}{3.4}$ 0.73

.38

.015 $\frac{1.5}{0.9 \times 2.6}$ $\frac{15}{23.4}$ 0.65

.045 $\frac{12.5}{23.4}$ 0.53

3.5 4 140

$\frac{3.5}{0.9 \times 1.1}$ 3.5 $\frac{3.5}{.9 \times 2.77}$ $\frac{4}{1.1 \times 2.77}$

5.75 5.75

7-10-36°c

6/10/24 $Na^+ + K^+$ ATPase

		Tr	/ Abs
H I M	1 Na	38	/ 0.42
	2 K	36	/ 0.445
H I M	3 Na	35.5	/ 0.45
	4 K	35	/ 0.455
H II F	5 Na	44	/ 0.36
	6 K	43	/ 0.365
H II F	7 Na	44.5	/ 0.35
	8 K	41	/ 0.385
H III R	9 Na	38	/ 0.42
	10 K	37	/ 0.43
H III R	11 Na	37.5	/ 0.425
	12 K	37	/ 0.43
B' I M	13 Na	54	/ 0.265
	14 K	51.5	/ 0.29
B' I M	15 Na	53	/ 0.275
	16 K	51.5	/ 0.2875
B' II F	17 Na	56	/ 0.25
	18 K	54.5	/ 0.265
B' II F	19 Na	56.5	/ 0.2475
	20 K	55	/ 0.26
B' III R	21 Na	55	/ 0.235
	22 K	53.75	/ 0.27
B' III R	23 Na	62	/ 0.21
	24 K	54	/ 0.2675
B (I+II) D	25 Na	55.5	/ 0.23
	26 K	54.5	/ 0.265
B (I+II) D	27 Na	59	/ 0.23
	28 K	54	/ 0.27

B Lower (H₂O) 100 / 0.0

1 M	50.5	/ 0.305
2 M	24	/ 0.62
3 M	12.5	/ 0.9

540 nm

odd Na^+
Even K^+

7/10/24 (Arrolein) 540 nm
(2+5+1.5)

B' I M	.04	75.5 / 0.1225
	.08	59.25 / 0.2275
B' II F	.04	77 / 0.115
	.08	63 / 0.20
B' III R	.04	82 / 0.0875
	.08	71 / 0.15
B (H II) D	.04	76 / 0.12 *
	.08	64 / 0.195

Blank	100 / 0.0
50 µg	88 / 0.055
100 µg	82 / 0.0875
150 µg	74 / 0.13

$Na^+ K^+$ ATPase

H I M	0.3	/ 0.06	µM/mg/hr
H II F	0.636	/ 0.09	µM/mg/hr
H III R	0.16	/ 0.08	µM/mg/hr
B' I M	0.63	/ 0.30	µM/mg/hr
B' II F	0.65	/ 0.55	µM/mg/hr
B' III R	1.8	/ 2.85	µM/mg/hr
B (I+II) D	→ 1.4	/ 1.6	µM/mg/hr

~~Blanke~~ (TNB)
Bleu (Azur A)
Rouge (Red 0)

1 Gm

- Na .25
- K .25
- Li .25
- Cs .25
- Ca .05
- Mg .05

Blanc
- Ach .25
- Chol .25
- GABA .25
- Gly .25
- Glu .05
- ~~Asp~~ .05
- NA .05
- Sero .05
- Pro .05

- Lavecchi · 1 mc + 1 mc tryptone
- Trichloroacetique · 1 mc + 1 mc tryptone

Tryptone Blanc

390
400
410
420
430
440
450
460
470
480
490
500

7/10/24 5 ml B(I+II)D + 7.5 ml TNS
+ 37.5 ml Imyfm
2.5 ml + 5 ml Sol. - 2 Imyfm

Excitation 365 TNS

Free Na Chcl Ach K Gly GABA Cs

(2x1) range

Free	Na	Chcl	Ach	K	Gly	GABA	Cs
29.5	33	33.5	31	33	28.5	28	31
42.5	47	44.5	39.5	37	37	34	40
67.5	76	71	62	68	61	60.5	65
81	88	79.5	69	73.5	70	69	74
72	82	75	65.5	69.5	65	64	72.5
59	71	63	54.5	63	56	55	63.5
45	56	49	45	50.5	44	41	52
35	44	38	34	42	33	34	42
25.5	33	29	26	32.5	24	24	32
18.5	25	21.5	19.5	25	18	18	24.5
13	18	15.5	14	18	12.5	12.5	17.5

(2.3 + 0.5 ml)

71.2 μM

TNS.

Protein 230 μg/ml

Salt (Na K Li Cs 50 mM
Ca++ Mg++ 10 mM)

390
Start

Max

$3 \times \frac{1}{10} (75.5$

High scale $\left(3 \times \frac{1}{10} (30 \right.$

$\left. 1 \times 1 \quad (75 \right)$

A 90

~~Free~~

Free Ach Li Oro | Mg++ Ca++ S Asp

Free	Ach	Li	Oro	Mg++	Ca++	S	Asp
24	27	28.5	24.5	43	43	62.5	63
36.5	39	43	36	57.5	57	65 (1x1)	68 (1x1)
58	63.5	68	60	91.5	93	45 (3x 1/10)	49 (3x 1/10)
65	68.5	82	66	60 (1x1)	60 (1x1)	59.5	66
56.5	64.5	80	65	60.5 (1x1)	61 (1x1)	65	75.5
49	56	66	53	57 (1x1)	56 (1x1)	65	76
38	43	56	41	88.5 (2x1)	92 (2x1)	58	70
33	34	44	32	74 (2x1)	74 (2x1)	50	69.5 ?
24	26	35	25	61 (2x1)	61 (2x1)	42.5 (3x 1/10)	52
18	19.5	25	17.5	46.5 (2x1)	46.5 (2x1)	84 (1x1)	101 (1x1)
12.5	14	18.5	12.5	34.5 (2x1)	34 (2x1)	60 (1x1)	77 (1x1)

340

	Glu	Nordmahn
2×1	67	63
1×1	73	68
3×1/10	51	48
	73	64
	61.5	72
	84	71
	76	66
	67.5	56
	56.5	48
	45	37
3×1/10	34	28.5
(1×1)	82	(1×1) 69

$$\left.\begin{matrix} 4 \times 1 \\ 60 \end{matrix}\right\} = 420$$

7|10174

390-470

I KSH EG 390-430

1 Free 4x1 70 Dry. 1x1/3 76 (3x1/10 31)

2 Free 4x1 61 Klebsiella 61

II KSH EG.

3 Free 4x1 61 Karpachi 2x1/10 60 (.1)

4 Free 4x1 61 Trefano 2x1/10 33 (56 3x1/10)

Exakt 390

Specht Emulsion (HNS)

Free	12 2N HCl 4 2-5 me	.05 Karpachi	.01 Trefano	(3x1/10 range)
5.5	9.5	14	13	
6	16	24.5	19	
6.5	26.5	42	29	
7	40	65.5	43	
8	51	84	54	
8	57	96	61	
8	59	97	61	
7.5	53	90	56.5	
7	46	75	48	
7	38	61.5	39	
6.5	31	47	31	
6	23	36	24.5	
5.5	18	26	19	
550 5	14	19.5	15	

Exakt

	1	2	3	4
250	4.5	6	6	6
60	5	7	6	6
70	5	6.5	6.5	7
80	5.5	12	7.5	8
300	6	16.5	7	8
10	6	19	7	8
20	5.5	16.5	7	8
30	5.5	20.5	8	9
40	6	25	9.5	11.5
50	6.5	33	15	16.5
60	6.5	39	25	22
70	7	44	42	30
80	7	47.5	50	36
400	8	53	58	43
10	8	55	60	44
20				
30	8	51	56	40
40	6.5	36	41	27
50	5.5	20	22.5	15
	5	11	12	10

500 / 22.4 → 2210, 1983, 2470 — 179

318 / 187.5 → 1305, 11250, 1800 — 169

.733 / .482 — $\frac{.25 \times 20 \times 10^{-4}}{3 \times 9.375} \div \frac{5 \times 10^{-4}}{27.9}$

9.375 / 6.25

.469 / .310 — $\frac{.159 \times 20 \times 10^{-4}}{3 \times 6.25} \div \frac{3.18 \times 10^{-4}}{18.75}$

6.87

.959 / .377 — $\frac{.582 \times 20 \times 10^{-4}}{3 \times 6.87} \quad \frac{11.64 \times 10^{-4}}{20.61}$ — 20.61 | 1164 | 56.4 — 1030.5, 133.5, 123.84, 9.84

.342 / .155 — $\frac{.187 \times 20 \times 10^{-4}}{3 \times 3.33} \quad \frac{374}{10}$

3.33

2.7

.268 / .137 / .432 / .066 — $\frac{.131 \times 20 \times 10^{-4}}{3 \times 2.7} \div \frac{262}{8.1}$ — 190, 243, 162 — 323, 280

$\frac{.366 \times 20 \times 10^{-4}}{3 \times 2.08} \quad \frac{732}{6.24}$ — 1080, 624, 4560, 4368, 1920 — 117.3

2.08

$\frac{\Delta \text{Abs./ml}}{1.36 \times 10^{4}} \times \frac{20 \times 10^{-4} \times 26}{\text{mg mole}} \times \frac{1}{3}$

2.6 ml (1 ml each)

7/10/21

Achondroplasia

Transmillion 412

	0	30	60	90	120	150	180
I HM	33 .482	30	27.25	25	22.5	20.25	18.5 .733
II HF	49 .318	46	43	40.5	38	36	34 .469
III HR	412 .377	33	25.5	20	16.25	13.5	11 .959
I B'M	70 .155	65	60.5	56.5	52.5	49	45.5 .342
II B'F	73 .137	69.5	66.25	63	60	57	54 .264
III B'R	86 .014	74	64	56	48.5	42	37 .432

Balance mainly NODTNs to 100%.

① I HM : 17.9 //

II HF 16.9 //

III HR 56.4 //

I 'BM 37.4 //

II 'BF 32.3

III 'BR 117.3

6/10/74

Wistar Albino

.4 + .4 + .2 + (.05 ml mmb)

NADH Diaphorase

	0	30	60	90	120	150	180
H I M	18	23	28	31.5	34.5		
H II F	18	21.5	24.5	28	31.5		
H III R	15.5	20	24.25	28.5	32.5		
B I M	20.5/21	24.5	28	32	35.5		
B II F	21.5	24.5	27.5	31.25	35		
B III R	22.5	25.5	28.5	31.5	35		
B(I+II)D	21	25	28.5	32	36		

Exchanger 490
counterflow (1×1 m)

150	0
60	0.25
70	0.25
80	2.5
90	12
500	20
10	41
20	61
25	65
30	64
40	55
50	42.5
60	32.5
70	22.5
80	14
90	12
600	3.

10/10/74

Acridine orange

20 µM (400 µM in 5% + 9.1 ml buffer)

2.5 ml was added cells in Media
added buffer in Blanks

~~Blank~~ ~~Media~~
~~1~~ ~~2~~
~~Cells~~ ~~Cells~~
~~3~~ ~~4~~

~~3 ml buffer~~
~~3 ml media~~

Blank Specia — 1 ml media
Exwahi Specimen (5 x 1 rays) — 1 ml buffer

Exwahi 292

220	1.5
230	2
40	4
50	11
60	31
70	44.5
80	49
90	64
300	59
10	35
20	17
30	6.5
40	3.5
50	5.5
60	10
70	16
80	22.5
90	42
400	58
10	78.5 (43) (4x1)
20	58 (4x1)
30	80 (4x1) (44) (3x1)
40	65.5 (3x1)
50	97.5 (3x1) (52.5) (2x1)
460	52 (2x1) 43.5 (1x1)
470	57 (1x1)
480	61 (1x1)
490	66.5 (1x1)
500	53 (1x1)
510	53 (2x1)
520	51 (3x1)
530	36 (4x1)
540	7.5 (5x1)

Exwahi Specn (5 x 1)

310	9.5
20	2.5
30	2.5-3
40	2
50	1.5
60	1
70	1
80	0.5
390	0.5
400	0.5
10	0.5
20	0.5
30	0.5
40	0.5
50	0.5
60	0.5
70	0.5
80	1.5
90	5
500	18
10	43
20	63.5
~~25~~ 30	67.5
50	66.5
40	56.5
50	44.5
60	34
70	24
80	16.5
590	9.5
600	4.5
10	2
20	1
30	0.5
40	0.5
50	0.5

Exakt
490

nm	(1/λ) m
505	67
510	41.5
515	47
520	59
525	68
530	73
535	72
540	68
550	53
560	40
570	27.5
580	17.5
590	9
600	4.

Mlmb added

Maxam 530

Emuthin 530 (5x1 range)

Excitation spectra

220	2.5
30	3
40	4.5
50	12
60	53
65	60
70	52
80	42
90	62.5
300	90
10	67
20	40
30	23.5
40	17
50	18.5
60	22
70	28
80	37
90	51
400	69
10	90 (49 (4x1)
20	65 (4x1)
30	88 (4x1) 48 (3x1)
40	69 (3x1)
50	99.5 (3x1) 54 (2x1)
60	82.5 (2x1) 44 (1x1)
70	60 (1x1)
80	64 (1x1)
90	73 (1x1)
500	70 (1x1)

510	60 (1x1)
520	over 100 (1x1)
530	over 100 (1x1)
540	67 (1x1)
550	2.5 (1x1) 24 (5x1)
560	8 (5x1
570	5 (5x1)
580	4 (5x1)
590	3 (5x1)
600	3 (5x1)

Excitation 300

Emission spectra (5x1)

380	59
90	45
400	36
10	28
20	23
30	19.5
40	17
50	15
60	13
70	12
80	10
90	11
500	19
10	39
20	70.5
25	86
30	90.5
35	91
40	86
550	70
60	54
70	39
80	30
90	65
600	over low
610	over low
620	93
630	15
640	4.5
650	3.5

1	2	3	4
490 - 525	490/530	490/530	490/530
70 (1x1)	73 (1x1)	74 (1x1)	86 (1x1)
285 - 525	300/530		
* imp 53 (5x1)	96 (5x1)	285/525	295/525
7.5 (5x1)		~~4~~ 3 (5x1)	94 (5x1)
		~~5~~ 7.5 (5x1)	

GABA Ach no effect on Blank.
GABA' increased (2) by 10%.

12/10/74 Pyrene 1mg/ml + 2ml meth + 15ml buffer

(2) well added 5mm. Blank add 0.1"

Chem

Ach (2)	1"
Ca	.5
Triton	.05

Dry Curve (4) Compressive treated alum

Dry specimen 5x1 mm

350	Crushed
60	24
70	34
80	50.5
90	71
	85.5
	90
	88
	82
	80
	80
	83.5
	88.5
	85.5
	79
	70
	60
	48.5
	37.5
	28.5
	21.5
	16
	12
	7.5
	4 - 590

Pyr 1x1 range

Exatahi max 335
Emmi max 395

Emmi 395
Exatahi speed (1x1) rng

250	4
55	5.5
60	8
65	11.5
70	16
75	17.5
80	14.5
85	11.5
90	11
95	11
300	14
05	19
10	25
15	34.5
20	44
25	54
30	65
35	82
40	66.5
45	31.5
50	13
55	7
60	5.5
65	5
70	5.5
75	23
80	overlap
85	
90	
95	
400	

Exatahi 335
Emmi speed

350	79
55	12.5
60	8
65	19
70	42
75	61.5
80	74
85	84.5
90	90
395	86.5
400	73.5
05	59
10	49.5
15	44
20	38
25	32.5
30	27.5
35	25
40	25
45	25.5 (26)
50	26.5
55	26.5
60	27
65	27.5
70	28
75	27.5
80	26
85	24
90	22
95	19.5
500	17.5
05	15.5
10	13.5
15	12
20	10
25	9
30	7
	6
	5
550	4 9.5

Exatahi 335

Blank 1	+1.5 Baff 1	+0.5 Atk 1	+0.5 Ca++ 1	+0.5 Trifluo 1
	(1)	(2)	(3)	(4)
350	53	66.5	66.5	17.5
60	7	8	18	2.5
70	37	42	41	3
380	69	75	72.5	4
340	82	85.5	83.5	4.5
395	79.5	82.5	80	5
400	68.5	71.5	69.5	4.5
410	47.5	48.5	47.5	4.5
	36	37.5	36.5	4
	26	28	27	4.5
	24	26.5	25.5	5
	26	29.5	28	6
	27.5	31.5	30	7
	29	34	31	7.5
	27	31.5	30	7
	24	27.5	25.5	6.5
	19	22	21	6.25
	15	17.5	16.5	5.5
	11	12.5	12	3.5
	7.5-8	9	8.5	3.25

(4) (.01 heater ohms)

Dmy. in

Exhin 335

4 x 1 nge

315	avalon
60	20
70	28.5
	34
	45
	42.5
	36.5
	36
	37
	41.5
	47.5
	53
	59
	64.5
	69.5
	61
	55.5
	45
	39.5
	32
	25
	20.5
	15
	10
	5.5
	3 - 600

{ 340 exh
(67.5)

Yuk	Ca 5 (2-5 m)	Tryptic .01 (53 mL)	
2 II	3 II	4 II	(excitatory Smythies 340 % of ctrl 5%)
(1x1 nyc)	Excitatory 335		(1x1 nyc)
350 26·5	27.5	27·5	350
7	6·5	3	360
40	35	5	70
73·5	60	7	80
90	72·5	8.25	90
71·5	58	8	
50·5	41·5	6·5	
35	32	6	
27·5	24	6	
26·5	23	6·5	
29	24·5	7·5	
31	24·5	8·5	
32·5	25	9·5	
31	22·5	10	
26·5	19·5	9	
22	15	8·5	
16·5	11·5	7·5	
13	8·5	7	
9	6	5	
6	4	4	
4	3	3	

Whole Rat brain Prep/fn · PNP · 4 Tris HCl (7.5 ml)
Homogenised in 80 ml conc. → keep homogenate
3500 rpm 1' PND.
Supt to Pellet and Homo & keep.

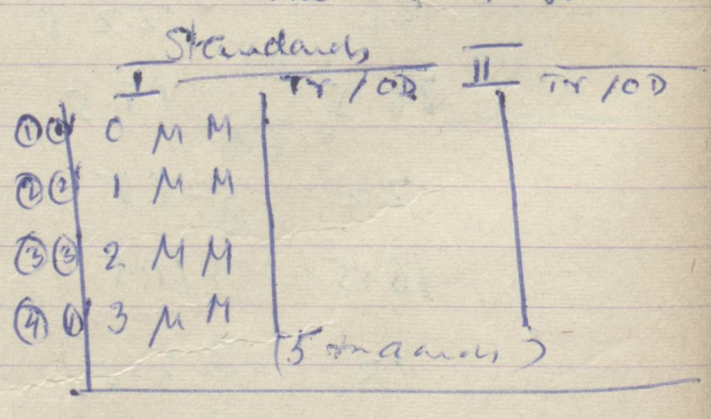
10,000 rpm · 10'
pellet → 30 ml buffer
keep for 2 hrs cold

Carp 40 | 28 ". from Fraction
Subcellular Cellular A, B, C fully &
made up to known volumes.

Method I Lowry Kamath & Wallach

Method II Lee BBA Skeletal muscle
(Sr. Procedure).

Remarks



I = [ ]

II = [ ]

DATE: 14/10/71

KILLED AT 10.00 AM

~~LYSING~~ Wt of tissue : 1.7 gms (3.4 gms) (2 normal rat)

Homogenate Volume: 90 ml + 1.7

PND Volume: 45 ml

L.S. Volume: 33 ml + Blackening 4 ml

Supt II Volume: 70 ml

A Volume: 10 ml

B Volume: 14 ml

C Volume: 12 ml

(L.S. kept in ice at 11.50 a.m.)

14/10/71

Assay Mixture Reagents

Method I 0.1 ml

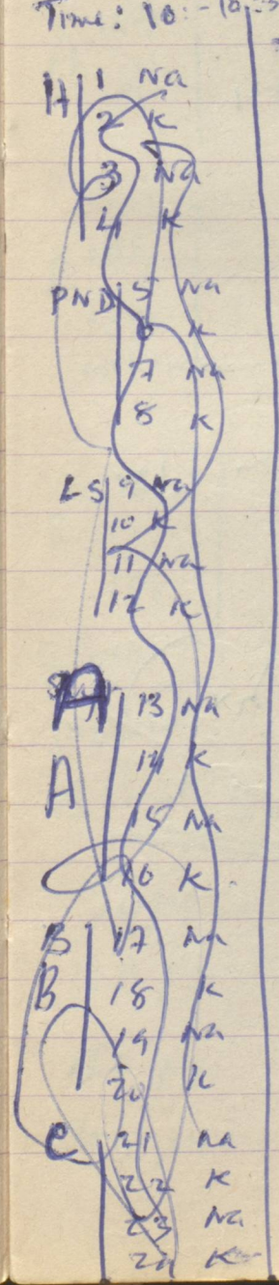
Method II 0.1 ml

Na - K ATPase I 540 mm

Na - K ATPase II

Time: 10 - 10:30

Time: Blank 10 : 10:30 30'



H 1	1 Na	40.5/0.39		64/0.195
	2 K	36.5/0.44		
2	3 Na	39.5/0.405		57.5/0.24
	4 K	36/0.44		64/0.195
3	5 Na	40.5/0.39		59/0.23
	6 K	37/0.43	0.1 ml	63/0.20
				56/0.25
	7 Na	44.5/0.35		
B 1	8 K	44.5/0.35		65/0.19
2	9 Na	47/0.33		62.5/0.205
	10 K	44/0.36		66.5/0.18
3	11 Na	47.5/0.325		62.5/0.205
	12 K	46/0.335		64/0.195
Std	25	0.0		66/0.18
				100/0.0
	26	1 μM		47/0.33
	27	2 μM		24/0.62
	28	3 μM		11/0.95

0.1 ml

Protein — Total

Crude H - ~~3.125~~ 6.25 mg/ml - ≃ 562.5

DND - 3.2 mg/ml — ~~312.5~~ 157.5

L S - 3.8 mg/ml — 126

Supt II - 1.9 mg/ml — 140

A - 2.1 mg/ml — 21

B - 1.9 mg/ml — 26.6

C - 1.2 mg/ml — 14.4

Black mg - 2.0 mg/ml — 8.0

Na-K ATPase — Kamat — nature

Crude ~ 0.45 μM/mg prot/hr — ~ 0.45 μM/mg prot/hr

~ 1.0 μM/mg/hr — ~ 0.8 μM/mg/hr

5' nucleotidase

H - Crude 2.2 ~~2.1~~ μM/mg prot/hr (1.1 μM/mg prot/hr)

B - Mouse ~~2.5~~ 4.8 μM/mg prot/hr. 4.8

DND 2.4 μM/mg prot/hr

LS 3.94 μM/mg prot/hr

~~Supt~~

A 1.14 μM/mg prot/hr

C 3.5 μM/mg prot/hr

BSS1 14/10 → 0.1 ml

5210 rpm

15/10/74 Protein [2+5+0.5]

.04 H	77 / 0.115	
.08	56 / 0.25	
.04 PND	81.5 / 0.09	?
.08	71 / 0.15	?
.04 LS	70 / 0.155	
.08	56 / 0.255	
.04 Supt II	85 / 0.07	
.08	75 / 0.125	
A .04	84 / 0.075	
.08	74 / 0.1325	
B .04	86 / 0.065	
.08	71.5 / 0.145	
C .04	91.5 / 0.04	
.08	80 / 0.095	.095
Blank .04 → .08	86 / 0.06	
Standard	74.5 / 0.13 (.125)	
Blank	100 / 0.0	

50 µg	89.5 / 0.0500
100 µg	82 / 0.0865
150 µg	77.5 / 0.11
200 µg.	

5210 rpm

[1.2 + 2.5 + 0.05 + 5 + 1.2 + 3]

5' nucleohidase (.05 ml) .1 ml

Time: 8:05 8:35

H 1	67.5 / 0.17	
2	64 / 0.195	
PND 3	79 / 0.105	
4	81 / 0.095	
LS 5	64.5 / 0.19	
6	65.5 / 0.185	
A 7	93.5 / 0.03	
8	94 / 0.0275	
B 9	77.5 / 0.11	
10	76 / 0.12	
C 11	79 / 0.105	
12	77.5 / 0.11	
Blank 13	100 / 0.0	
1 µm 14	~~77.5 / 0.11~~ 56 / 0.25	
2 µm 15	37 / 0.43	96 nucl. (.04 in prem)
3 µm 16	20.5 / 0.69	
Supt ~~II~~ 17		
18		

Remarks

* Equal time DTNB incubation

Start incubation when reading the absorbance.

Gmelt $\frac{.4}{3} \times \frac{20}{6.25 \times 16} \times 10^4$ H { : 40 μM/hr/mt

Mamb $\frac{.44}{3} \times \frac{20}{44 \times 10^4}$ ① B { = 166 μM/hr/mt

② : 200/μM/h/mt

PND	32 MM/hr/mt
LS	75 μM/hr/mt
SUP II	30 μM/hr/mt
A	32 μM/hr/mt
C	130 μM/hr/mt

0.4 ml Tris buffer 0.02 M 7.4 pH

0.4 ml NADH 2 mg/10 ml.

0.2 ml $K_2Fe_3(CN)_6$.

.05 ml mamb

$\frac{OD/ml}{0.22} \times \frac{21}{mg/mt}$ Wet Wt %

15/10/34

Acholinesterase 0.1 ml ~~2.6~~ 2.6 ml 412 mμ.

	0	30	60	90	120	150	180
H	35 .416	30	25.5	22	19.5	16.5	14 .554
PND	48 .319	45	42.5	40	38	35.5	33.5 .482
L S	39 .409	32	26	21.5	17.5	15	13.5 .870
SUPT II	59 .229	55	51	47.5	44.25	41	38.5 .414
A	60 .202	56 (57.5)	55.5	53.5	51.5	49.5	47.5 .324
B	52/.285	43.5	37	31	26.5	22.5	19.5 .725
C	51 .291	46.5	42.5	39	36	33	30.25 .523
NO DTNB	100	100	—				
NO Enzyme	72	72	72 (71.75 72.07)	71.75	71.5	71.5	71.25
(B) + NO Substrate	49.5	49	49	48.75	48.5	48.5	48.25
+ substrate	46 .111	36	29	23.5	19	15.5	13 .566

NADH Dehydrogenase: 60 units in 100% .05 methylene

15/10/34		30				Activity
H	20.5 .694	24	26.25 .578	28.75	31.5	65.36
PND	23 .634	24.75	26 .685	27	28	55.93
L S	22 .658	25	28 .552	31.5	35.25	94.17
SUP II	26.75 .570	28	28.5 .545	29.25	30	44.41
A	36.5 .438	36.75	37 .432	37	37	96.47
B	22 .656	24.5	27 .569	29	32	158.1
C	28 .636	24	25 .602	25.5	26	101.3

Blank

Sivaramakarma's Cocktails

SDH

	0	30	60	90	120	150	180
H	41.5 .382	45	48	52	56 .252	0.95 85	
PND							
LS						0.7796	
SUP II	53.5 .272	54.5	55.5	56.5	57 .244		
A	47 .342	51	56.5	62.5	69 .161	3.704	
B	44.5 .362	47	50	53	56.5 .248	2.534	
C	50 .301	52.5	55	57.5	60 .222	ND	

Protein check

Standard (Mean) P_{H} 239

H	.02	79.5 / 0.1
PND	.02	89.25 / 0.05
LS	.02	83.5 / 0.075 – 0.08
Supt II	.04	85 / 0.07
A	.04	85 / 0.07 – 0.0725
B	.04	85 / 0.07
C	.04	86 / 0.065
Blank 2	.04	

Blank: 100 / 0.0

(Sharp
Excitation
335

360
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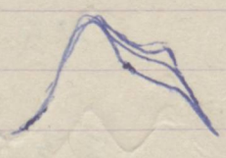


B(I+II) 6/ 4/10/24

4 ml + 0.5 mg Physeue.
Homopiral in 20 ml Ringer.

0.5 ml Salt + Ringer + 2.5 ml Ringer

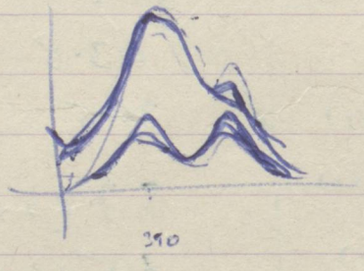
(maximum response)

① Buffer ② Ach ③ CaRS A ④ Asp. in free

Buffer (Resp) Blue	Ach	CaRSA	Asp
9 (1×1)	11 (1×1)	10 (1×1)	5.5 (1×1)
20.5	21.5	20	22
42	43.5	42.5	40
64	65	63.5	59
75.5	77	75.5	71
83.5	85	82	77
87.5	90	86.5	82
83.5	86.5	82	77
69	73	68.5	64.5
56.5	60	56	53
47	50.5	47	45.5
41	43.5	40.5	39.5
35 (1×1)	36.5	39	33
29.5 (1×1) 55 (2×1)	57 (2×1)	53.5 (2×1)	27 (1×1) 51 (2×1) 95.5 (3×1)
43 (2×1)	45 (2×1)	42 (2×1)	40 (2×1)
69.5 (3×1)	72 (3×1)	67.5 (3×1)	34 (2×1) 63.5 (3×1)
65	68	63	59
60	65	61	57
58	62.5	60	56
56.5	62	57.5	53.5
54.5	60.5	56	53
55	56.5	55	52.5
54	61	56.5	51
53.5	60.5	54.5	49.5
49.5	57	52	48.5

(3×1) →

360
up
mReps 2
5



	①	②	③	④	
485	49.0	53.5	49	46.5	
490	42	47.5	42.5	40	
495	39.5	43	39	36	
500	34.5	41	35	33	
505	31.5	34.5	31.5	29	
510	27	30	27.5	25	
515	23.5	26	24	21.5	
520	19.5	22.5	20.5	19	
525	17	19	17.5	16	(3x1)
530	14	16	15	13.5	
535	12	13.2	12	11	
540					
545					
550					

(Extrait 335⁻)

Bcc (1x1) ½ intervalle.

Bayer · GAMA · Gba - Largachi (chlorpromazine) .01

360	12 (1x1)	12.5 (1x1)	10.5 (1x1)	6 (1x1) (21 (3x1)
365	24.5	25	22.5	6
370	56	57	52	8
375	84.5	85.5	78.5	8.5
380	103 (1x1)			
385	67 (4x1/10)	68 (4x1/10)	63 (4x1/10)	9 (1x1)
385	71.5	72.5	103 (1x1) 67 (4x1/10)	9 (1x1
390	75.5	76	70	9
395	73.5	73.5	68	8 (1x1)
400	95.5 (1x1)	96 (1x1)	88 (1x1)	7.5
405	78.5	79	71.5	6.5
410	65.5	65	59	6
415	55.5	55	50.5	5.5
420	47 (1x1)	47	42.5	5 · 5.25 (1x1)
	88.5 (2x1)	88.5	80.5	10 (2x1)

Buffer	GABA	Glu	Lactate
73 (2x1)	72.5	65	10.5
4:30 59.5	59	52	11
35 52	51.5	45	11.5
49 (2x1)	56 (2x1)	43 (2x1)	12.5 (2x1)
90 (3x1)	92.5	80 (3x1)	23 (3x1)
45 92	94	78	25.5
50 91	95	77.5	26
55 91	93	76	27
60 91	94.5	76.5	27.5 – 28
65 93	97.5	78.5	27.5
70 94	98.5–99	79	25
75 93	96.5	76.5	24
80 86.5	90.5	73	21
85 83	86	68	19.5
90 73.5	77.5	62	16.5
95 70	73	56.5	15.5
5:00 62	65	51	14
05 55	56.5	44	11.5
10 47	49.5	39.5	10.5
15 40.5	41.5	34	9
20 34	35.5	29	8
25 30	30.5	25	6.5
30 23.5	24.5	20	5.5
35 20	20.5	16.5	4.5
41 16.5	17	13.5	4 – 5:40

47°

A ← 49

Kamat Balaani

$Na^+ K^+$ ATPase

H Cmcle. 16.10 - 0.57 0.32 0.02, 0.10

B. Memb 16.10 0 0 1.596 0.588

Memb 14.10 Dealyzed: 1.5, 1.5 1.15, 1 pM (new preparation required)

ATP 8.5 mg / 30 ml

AMP 22 mg / 20 ml

[B-14 Dealysed from 7.00 - 3:00 20 hrs.]

Date
16/10/74

Protein

Killed at 9:30

Wt of tissue		3: 5gms	
Homogenate Volume		75 ml	7.14 mg/ml
PND	Volume	45 ml	3.4 mg/ml
L.S.	Volume	33 ml	3.6 mg/ml
Synpt II	Volume	70 ml	2.05 mg/ml
A	Volume	6 ml	~~3.14 mg/ml~~ 1.7 mg/ml
B	Volume	14 ml	1.7 mg/ml
C	Volume	20 ml	1.24 mg/ml
Blank mg. Volume:		15 ml	3.65 mg/ml

B14D = 1.3 mg/ml

kept for analysis 10.15

7.730 37°C 540 mm. 30' 0.1 ml 540 nm 20'

Na - K ATPase (Kamat) .1ml — Na-K ATPase (Boltram) .1ml

H
1 1 Na 36.5/0.44 — 57/0.245
2 K 31/0.51 — 56.5/0.2475
2 1 3 Na 32/0.49 — 59/0.23
4 K 31/0.51 — 57/0.2425
5 Na 54.5/0.265 — 75.5/.1225

B
1 6 K 54.5/0.265 — 68/0.17
2 7 Na 54.5/0.205 — 74.5/0.1275
8 K. 54.5/0.265 — 71.5/0.145

B14D
Std. Blank
1 1 µM 9 Na 60/0.22 — 83.5/0.08
2 µM 10 K 55/0.26 — 78.5/0.105 - .1075
2 2 3 µM 11 Na 54.5/0.225 — 82/0.085
12 K 54.5/0.265 — 79/0.1025 - .105

Std		Blank	
Blank	100/0.0	1 µM	102/0.0
1 µM	43/0.375		48.5/0.315
2 µM	22/0.66		
3 µM	11/0.95		

Nucleotidase

Crude 1 4.47 μM/mg/hr
2 3.9 μM/mg/Hr

DND 5.2 μM/mg/hr

LS 4.82 μM/mg/hr

A = 2.9 μM/mg/hr

B = 5.6 μM/mg/hr

C = 9.6 μM/mg/hr

Supt II = 5 μM/mg/hr

B14D = 5.77 μM/mg/hr

check C. Protein.
Bradford Belo.

16/10/74 7.10 - 7.40

30' 540mm. 0.1ml

5' nicholam

H	1	31	0.51 X
	2	36.5	0.44
PND	3	51	0.29
	4	52.5	0.28
LS	5	54	0.265
	6	51	0.29
A	7	82	0.085
	8	84	0.075
B	9	71	0.15
	10	70	0.155
C	11	64.5	0.19
	12	65	0.1875
Rupi II	13	69	0.165
	14	69.5	0.1675
Smel Mark		~~80~~	~~0.11~~ 5 100 0.0
17			
16	1 µm	47	0.32
19	2 µm	24	0.615
20	3 µm	11	0.95
B14D	15	76.5	0.115
	16	75	0.125

(17/10/74 Mark)

Protein Check

Mark	100	0.0
B .04	88	0.055
.08	78.5	0.105

17/10/74 morning

Protein

H .02	79.5	0.105
.04	65	0.1875
PND .02	89.5	0.0475
.04	80	0.095
LS .02	88.75	0.0525
.04	78	0.1075
A .04	90	0.045
.08	80	0.10
B .04	89.5	0.0475
.08	79	0.1025
C .04	93	0.0325
.08	86.5	0.0625
Rupi II .04	86	0.065
.08	76.5	0.115
Mallory .04	76	0.12
.08	64	0.195
Mark B14D .04	92	0.0375
.08	85	0.07
50 µg	100	0.0
100 µg	90.25	0.045 (35)
150 µg	84	0.075
~~200~~ µg	89	0.105

C .04 93 0.0325

.08 85 0.07

A determinations

H : 33 µM/hr/mg/ml

PND : 20 µM/hr/ml

Ls = 62 µM/hr/ml

A = 24 µM/hr/ml

B = 88 µM/hr/ml

B_2 enzyme (OES)

C = 66 µM/hr/ml

SupII = 60 µM/hr/ml

Blank mix 66 µM/hr/ml

Blank mix

B14 DIALYSED 120 µM/hr/ml

OD/ml 21

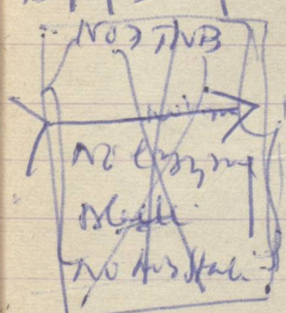
6.22 × mg/ml

1 ml enzyme
1 ml $K_3Fe(CN)_6$
1 ml

16/10/74 11:02 am

Achsneigung

	0°	30"	60"	90"	120"	150"	180"
H	22 .658	19	16.5	14.25	12.5	11	9.5 - 9.75 1.028
PND	25/27.5 .560	26.75	26	25	24	23	22 .654
LS	30.5 .516	27	24	21	18.5	16.5	14.5 - 15.0 .839
A	40.5 .392	39.75	36.75	38	37	36	35.25 .456
B_1	39.5 .404	36.5	33.5	31	28.5	26	24 .620
B_2	40 .398	37	34	31.5	29	27 - 26.5	25 .602
C	38.5 .414	36.5	34.75	33	31.5 →	30	29 .538
Fupt II	37 .432	34.5	32.25	30	28.25	26.5	25 .602
B/HD	38 .429	34.75	31.75	29	26.5	24	22.25 .658
	24.5 .611	21	18	15.75	13.5 .137	12	10.5 .979



17/10/74 NADH Diagram

	0	30	60	90	120	150	180
H	17 .770	20.5	22.5 .646	25	27.5		57.69
PND	19.5 .710	21	21.5 .668	22	22.5 .22.75		41.7
LS	20.5 .688	22.5	24.630	25.5	27		63.8
A	24.5 .611	26.5	28.5 .545	30.5	33		131.4
B	23.0 .638	24.5	25.5 .593	26.5	27.5		89.37
C	23.5 .624	24	24 .620	24	24.25		24.56
Fupt II	24.5 .611	25	25.75 .569	26.25	27		36.22
Blue mj	14 .724	22	25.5 .593	26.5	31.5 →		135.9
B/HD	23.5 .624	24.5	25 .604	26	27		NOT Cal'd

Achsneigung

150	180
2.5	57.69
	41.7
	63.8
	131.4
	89.37
	24.56
	36.22
	135.9
	NOT Cal'd

(Enzyme kept on ice for 1')

Assay 15" at 0.05 mg enzyme

$\frac{\text{OD/min} \times 1000}{10.6 \times \text{mg protein}}$ = U/mg milligram

$\frac{\text{OD/min}}{5.11} \times \frac{1000}{\text{mg/ml}}$ = U/ml milligram

Trypsin added.

Trails	0	15"	30"	45"
L-14	54	65	50	88
L-15	51.5	67.5	83	
YL-16	49	50.5	52.00	53.5
YL-17	50	51	52.5	54
Y1+16	48.75	48.75	49	49
Y1+17	—	—	—	—

283 x 10 2

SDH

	0	30	60	90	120	
H	42.5 .372	45	47.25	49.25	51.25 .290	1.069
PND	44 .357	44.25	44.75	45.75	46.5 .332	0.6808
LS	47 .328	48	49.5	51	52.5 .260	1.237
A	49 .310	51	53.5	56.25	60 .222	4.79
B	48.25 48.5 .314	48.5 48.75	49.25	49.75	50.5 .296	0.9806
C	48.25 .316	48.25 48.5	48.5 48.75	49	49.25 .308	0.5974
Supt II	54.5 .264	55.5	56.5	57.5	58.5 .233	1.400
Blank	49.5 .372	45	47.5	50.5	54 .264	2.638
Tris						
BHD	49 .310	51.5	54.5	57.5	60.5 .219	

Singer & Kearney method (1953) modified by Swaminathan

Reagents

I. KCN 19 mg / 2 ml

2 ml neutralised by HCl & made up to 10 ml.

II Ca Cl_2 ~~0.5 M~~ (0.8 mM)

III DCI 6 mg / 80 ml (2,6 Dichlorophenol Indophenol)

IV P.M.S 10 mg / ml (Phenazine methosulphate)

V Succinate 0.5 M (Neutralised)

VI phosphate buffer pH 7.4 0.5 M diluted to 0.1 M

Cocktail

- 1 ml phosphate buffer pH 7.4 (0.1 M) (VI)
- 0.1 ml $CaCl_2$ 0.8 mM (II)
- 0.3 ml DCI (III)
- 0.1 ml Succinate (V)
- 0.1 ml KCN (I)
- 1.25 ml water

per assay. (1.85)

+ 0.05 ml PMS

+ 0.1 ml enzyme

The read at 600 nm - water blank

17/10/74

μMOL/NPnmol/mlt/mg

μM/p cited/mlt/mg

1 μg = 6.4 klett

10 μg = 0.205 OD

Lysosomal enzymes

		Acid phosphatase 1 μg = 6.8 klett-units		Aryl sulfatase A 515 nm	
A	H 1410	245	12.48	0.23	0.3625
B	B 1410	230	32.1	0.195	0.8052
C	H 1610	310	11.44	0.27	0.3117
D	PND 1610	118	9.191	0.23	0.5294
E	SUPT 1610	105	13.54	0.06	0.2304
F	A 1610	120	16.68	0.075	0.3471
G	B 1610	140	21.77	0.125	0.5794
H	C 1610	78	16.62	0.19	1.2

30 mg 0.5 ml

Standard Stock

x 1/10

30 mg 0.2 ml

x 10

ATP 3 hr

SDH : $\frac{OD}{min} \times \frac{1000}{\text{moles added}}$.

H → 1 μM/mg/hr 1.3 μM/mg/hr

Below 0.3 μM/mg/hr.

B → 0.8 μM/mg/hr 1.6 μM/mg/hr

Below 0.4 μM/mg/hr

C → 5.4 μM/mg/hr 6.6 μM/mg/hr

Below 0.3 μM/mg/hr

18/10/74

Adult male 2 rabi

Tissue wt 3.5 gms

Homogenate	92 ml	6.12 mg/ml
PND	53 ml	4.4 mg/ml
LS	35 ml	5 mg/ml
SUPT II	75 ml	1.8 mg/ml
Black ring	4 ml	6.12 mg/ml
A	6 ml	2.2 mg/ml
B	15.5 ml	2.5 mg/ml
C	16 ml	1.6 mg/ml

0.1 ml 30' Kamal. 352-422

Na-K ATPase

H	1	18.5/0.73
	2	15/0.825
	3	21/0.68
	4	15.5/0.81
	5	25/0.60
B	6	23.5/0.63
	7	27/0.58
	8	23/0.655
C	9	62/0.2075
	10	46.5/0.33
	11	67/0.175
	12	45/0.335

Stand.

25 Blank 100/0.0

26 1 µM 50/0.30

27 2 µM 26/0.585

0.1 ml 30' Balaram 352-422

Na-K ATPase

	13	17/0.773 Discarded
H	14	64.5/0.19
	15	71/0.15
	16	65/0.1775
	17	77/0.115
B	18	75/0.125
	19	77/0.115
	20	73/0.135
	21	87/0.06
	22	87/0.06
C	23	87/0.06
	24	85.5/0.0675

25 Standard. Blank 1.

29 1 µM 50/0.30

30 2 µM 27.5/0.56

ATP Conc. tripled.

H = 4.1 μM/mg/Hr

DMJ: 4.3 μM/mg/Hr

LS: 5.2 μM/mg/Hr

SVPII: 4.8 μM/mg/Hr

A: 3 μM/mg/Hr

B: 7.5 μM/mg/Hr

C = 11.25 μM/mg/Hr

Blank: 4 μM/mg/Hr

5' micrometer 3.58 - 425

H	1	40 / 0.395"	
	2	40 / 0.395"	
PND	3	51 / 0.29	
	4	52 / 0.285"	
LS	5"	39.75 / 0.40	
	6	39 / 0.405	
SUP II	7	74 / 0.13	
	8	74 / 0.13	
A	9	79.5 / 0.10	
	10	79 / 0.1025"	
B	11	52.5 / 0.28	
	12	52 / 0.285"	
C	13	52.5 / 0.28	
	14	53 / 0.275"	
Black	15	44.75 / 0.35"	
my	16	41 / 0.385"	
m Standard	0	100 / 0.0	
12	1 μm	50 / 0.30	
14	2 μm	26.5 / .575	
20	3 μm.	13 / 0.575 - 0.9	

0.1 mil engine

Molins

.02	H 1	80.5 / 0.095"	
.04	2	64.5 / 0.1925"	
.02	PND 3	85 / 0.075"	
.04	4	74 / 0.13	
.02	LS 5"	83 / 0.0825"	
.04	6	69.25 / 0.16	
.04	Sup II 7	83 / 0.0575"	
.08	8	76.71 / 0.115"	
.04	A 9	85 / 0.07	
.08	10	73 / 0.135"	
.04	B 11	82 / 0.085	
.08	12	68.5 / 0.165"	
.04	C 13	87.5 / 0.055"	
.08	14	78.5 / 0.11	
.02	Black 15	79.25 / 0.10	
.04	my 16	70.5 / 0.15"	
Black	17	100 / 0.0	
50	18	90.25 / 0.045"	
100	19	85 / 0.07	
150	20	78 / 0.1075"	
200	21	69.5 / 0.16	

Acheuléen

H 44 µM/m/mL

PND 24 µM/m/mL

LS 85.4 µM/m/mL

A 32 µM/m/mL

B { 128 µM/m/mL
  { 156 µM/m/mL

C → 93 µM/m/mL

Supt → 56.8 µM/m/mL

Blackwing → 60 µM/m/mL

	SDH	mg	µMol/mg Prot/mL
H	1.104	$\times 10^{-2}$	
PND	0.4238	$\times 10^{-2}$	
LS	0.9145	$\times 10^{-2}$	
Supt II	— ND —		
A	2.4	$\times 10^{-3}$	
B	0.8136	$\times 10^{-2}$	
C	— ND —		
Blackwing	1.043	$\times 10^{-2}$	

2·3 + 0·1 + ·1 + ·1
Lysyr mas dilute

A. Chromatin

18/10/14

	0	30	60	90	120	150	~~180~~
B_1	54 ·264	44·5	37	30·5	25·5	21·5	18 0·745
B_2	52 ·254	40·5	32	25·5	20·5	16·5	13·5 0·870
H	39 ·409	33·5	28·5	24·5	21	18	15·5 0·810
PND	48·5 ·314	45·5	42·5	40	37	35	33 0·452
LS	40 ·328	32	26	21	17·5	14·5	11·5 ·440
A	72·5 ·140	69·5	67·0 -66·5	64	61·5	59	56·5 ·245
C	60·5 ·249	55·5	51	47·75	43	39·5	36 0·444
Sup II	70·5 ·152	66·5	62·5	58·5	55	52 -51·75	49 ·310
Blank mj	36·5 ·408	28·5	22·5	18	14·5	11·5	9·75 -9·5 1·023

18/10/14

SDH

(0·1 ml added)

	0	15	30	45	60	
H	35·5 ~~·450~~	37	38·5	40·25	42	·450 – ·377
PND	34	34·25	34·75	35·25	35,75	·469 – ·453
LS	32·5	33·5	34·5	35·5	36·5	·489 – ·438
Sup II	Nil					
A	36	37	38	39·5	41	·444 – ·387
B	34·75	35	35·5	36	36·5	·480 – ·438
C	Nil					
Mixture M	33·5	34·5	36	37·5	39	·478 – ·409

NADH dehydrogenase — µMoles / mg prot / mt

H	37.27	milk inhib.
PND	35.77	milk inhib.
LS	55.17	milk inhib.
A	187.3	milk inhib.
B	101.2	milk inhib.
C	18.79	milk inhib.
Sup II	43.23	milk inhib.

NADH 0.1 ml mins ✓

Cycle	0	30	60	90	120
H 1810	14.5 .639	17	19.5 .710	22.25	25
DND	17.5 .757	20	21.5 .668	23	24.5
LS	15 .824	18	21.5 .665	24.5	26
A	24 .620	33	41 .367	41.5	46
B	18 .745	21.5	25 .602	28.5	32
C	19.25 .710 -19.5	19.75 -20	20.75 .693	21.75 -22	22.75
SUP II	27.5 .560	29	30.25 -30.5 .516	32	34

Non linear →

Blank water 340 nm U.V

Pyrene 25 mg + 2 ml 100 µ s/m BSA
+ 8 ml Buffer.

Pyrene 25 mg in 5 ml Hexane

Chloroform and Benzene in Hexane

Hexane Blank	Hexane Blank	BSA Blank	BSA Blank	check
345-390	345-390	332-390-470		
50	50	75-43	77-46	All 3 x 1 ml.

1 Hexane

2 + Drugs in Hexane 1 ml

3. BSA + Drug 1 ml

Maxima reading

345-390 -470	345-390	332-390-470	332-390-470
58-13.5	57-13.	83-46	30-20

Irr-KATP

Control

H = 0.8 µg/ml 1.1 µM/ml

Balance

0.3 µM

B: 1 1.2 0.17 .25

19/10/74 Monkey brain

cf 21/10/74 Grey Cortex in left hemisphere

weight starting sample 4 Gms

Homogenate	1mm	85 ml	6.25 mg/ml
DND	1mm	45 ml	5 mg/ml
LS	~~Volume~~	33 ml	3.6 mg/ml
Sup I - II	1mm	80	1.2 mg/ml
A		6 ml	1.5 mg/ml
B		10 ml	3.8 mg/ml
C		16 ml	0.9 mg/ml

37°C

Na - K ATPase 0.1 ml 540 mμ 0.1 ml

Reaction 30' Balance 30'

(ATP conc: 3 times molar)

1	H	Na	17.5 / 0.76	9	Na	64 / 0.195 ✗
2		K	14.5 / 0.83	10	K	65 / 0.185
3	H	Na	16.2 / 0.79	11	Na	67.5 / 0.1725 ✓
4		K	12.5 / 0.9	12	K	63 / 0.20
5	B	Na	16.75 / 0.77	13	Na	77.5 / 0.11 ✓
6		K	15 / 0.825	14	K	76 / 0.12
7	B	Na	16.2 / 0.79	15	Na	76.75 / 0.115
8		K	14 / 0.85	16	K	74 / 0.13

17 Blank 100 / 0.0

18 1 μM 47 / 0.33

19 2 μM 25.5 / 0.55

20 3 μM 14 / 0.875

Specht

Hexane

① Exakthin (3×1)

Emax = 3~~87~~ 387

3×1 mm

250	1
55	1
60	1
65	1.5
70	2.5
75	9
80	16.5
85	22
90	23.5
95	21
300	14
5	9
10	7
15	5.5
20	5.5
25	6.5
30	5.5
35	28
3340	56.5
345	61.5
345	61.5
50	51
55	40
60	33
65	24
70	17
75	14.5
80	20.5
85	24

Exaktin 345

350	10	
55	8.5	
60	9	
65	15.5	
70	30	
75	43.5	
80	56.5	56
85	61	62
390	62	62
5	55	55.5
400	49	44.5
5	41	42
10	34.5	36.5
15	29.5	31.5
20	26.5	
25	22	
30	19	
35	17.5	
40	17	
45	16.5	
50	15.5	
55	15	
60	15	14.5
65	14.5	14
70	14.5	14
75	13.5	13.5
80	12.5	12.5
85	11.5	11.5
90	10	10.5
95	9	9.5
500	8	8.5
		7.5 (550)

② Hexane

BSA

3×1

Zwölf Blatt

3x mm

Emax = 390

Exaktin 332.5

250	4	
55	5.5	350 13.5
	8	55 5
	12.5	60 10
270	15.5	65 38
	13.5	70 68
280	8.5	75 91.5
85	7	80 93.5
90	5.5	85 93.5
95	11.5	90 97
300	18	95 94
5	26	400 79
10	36.5	05 62
15	54	10 53
20	63	15 48.5
325	71.5	20 41.4
330	92.5	25 39
332.5	98	30 36
335	94.5	35 36
40	51.5	40 38.5
45	21.5	45 42
50	12	50 44
55	9	55 45.5
60	8.5	60 46.5
65	8	65 47.5
		470 47.5
		75 46
		80 44
		85 40
		90 37
		95 33
		500 30
		05 25.5
		10 21.5
		515 17.5

BSA + Dry

4×1

Dry treated

~~4~~ 1 mm

Emax 390

Exakt	Emax: Exakt: 335
250 1.5	
1.5	310 20
2	34
3.5	54
5	65.5
6	65.5
6	65.5
6.5	61.5
8	61.5
10	61.5
350 11.5	400 58.5
12.5	42
10 15.5	38
22	36.5
27.5	36
39	36
56.5	38
335 62.5	42
56	46.5
43	52.5
36	56.5
25	59
18.5	60.5
18	60
	57.5
	53
	46
	40
	35
	30
	20 (500

18/10/74 30' 0.1ml 540mm
5' Mclohdn

H	1	62.5/.205
	2	62.75/0.2025
PND	3	68/0.165
	4	69/0.16
LS	5	69/0.16
	6	70/0.155
SVP	7	87/0.06
	8	88/0.055
A	9	91/0.04
	10	89/0.05
B	11	66/0.18
	12	69/0.16
C	13	88/0.055
	14	89.75/0.05
15	Blank	100/0.0
16	1/μM	49/0.31
17	2/μM	25.5/0.59
18	3/μM	14/0.85

C 105+2 540mm
20/10/74 Cholin

H	.021	80.5/0.095
	.042	62.5/0.205
PND	.023	81.5/0.09
	.044	67/0.175
LS	.045	77/0.1125
	.086	57.5/0.24
SVP	.047	91/0.04
	.088	83/0.08
A	.049	88/0.0575
	.108	78.5/0.105
B	.047	72.5/0.140 (.1425)
	.088	52.5/0.28
C	.049	93/0.0325
	.088	87/0.06
Blank	15	100/0.0
50 μM	16	90/0.02475
* 100 μM	17	85/0.07
150 μM	18	72.5/0.14
200 μM	19	69/0.165

5' nucleotidase		Acetylcholinesterase
H	2.2 µM/mg/hr	17.4 µM/hr/mg
PND	2.2 µM/mg/hr	17.6 µM/hr/mg
LS	3.05 µM/mg/hr	26.27 µM/hr/mg
SVP	3.3 µM/mg/hr	20 µM/hr/mg
A	2 µM/mg/hr	19.5 µM/hr/mg
B_1	3.3 µM/mg/hr	42.7 µM/hr/mg
2	3.1 µM/mg/hr	47 µM/hr/mg
C	3.5 µM/mg/hr	22 µM/hr/mg

NADH Dehydrogenase		µmol/mg prot/min
H	58.57	millimg
PND	78.54	millimg
LS	113.4	millimg
Sup II	81.06	millimg
A	293.6	millimg
B_1	119.6	millimg
B_2	127.9	millimg
C	133.6	millimg

A-cholinesterase 2·3+·1+·1+·1

19/10/74

Sample	0	30	60	90	120	150	180
H	36.5 .438	34.25	32	30	28	26.5	24.75 .102
PND	40.5 .342	38.5	36.5	34.75	33	31.5	30 .523
LS	49.5 .314	46	43.5	41.25	39	37.25 37.75	35 .456
SVP II	65 .187	64	63.25	62.5	61.5	60.75	60 .222
A	68.5 .164	67.5	66.5	65.25	64	63	62 .208
B_1	43 .367	39	36	32.5	29.5	27	24.5 .611
B_2	44 .357	40	36	32.5	29.5	26.75	24.25 .620
C	67 .174	66.25	65.5	64.75	64	63.25	62.5 .205

20/10/74 340 nM NADH Diaphorase 0·1ml+·2+·4+·4

	0	30	60	90	120	150	180
H	11.5 .940	15.5	18.5 .733	21.75			
PND	12 .921	16.5	20 .849	22.0			
LS	15.5 .810	21.5	26.5 .571	30			
SVP II	25.5 .593	27.5	29 .535	30.25			
A	22 .658	31	39 .409	43.5			
B_1	13 .886	19	23.5 .624	25			
B_2	13 .886	20.5	24.5 .611				
C	20.5 .686	27.5	24 .620	27.5			

Inhalt Register.

21/10/74

Adenylate cyclase assay

PR

Tube Nos	(In marsha's handwriting)		
1	H 1410	1a 1b	
2	H 1610	2a 2b	
~~3~~	~~H 1610~~	3a 3b etc.	
3 4	H 1810		
~~5~~	~~H 1610~~		
4 ~~6~~	H 1910		
~~7~~	~~H 1910~~		
4 ~~5~~	B 1410	9	C 1410
2 ~~8~~	~~B 1610~~	10	C 1610
6 ~~10~~	B 1610	11	C 1810
7 ~~11~~	B 1810	~~12~~	C 1910
~~12~~	~~B 1810~~		
8 ~~13~~	B 1910		
~~14~~	~~B 1910~~		
—		13	C 1810 F
15	B 1810 F		
FREE 16	B 1810 F	14	C 1810 F
17	B 1810 Ach		
18	B 1810 Gln		

See ⇒ Next page

19	B 1810 Serotonin	
20	B 1810 GABA	
21	No enzyme	ATP Blanks
22	No enzyme	
23	H 1410	
24	H 1610	
25	H 1810	
26	H 1910	
27	B 1410	
28	B 1610	
29	B 1810	
30	B 1910	

Enhels

O'marsha's

31	C 1410
32	C 1610
33	C 1810
34	C 1910
35	C 1810 A
36	C 1810 Gln
37	C 1810 S
38	C 1810 Ach

AMP Standards

39	
~~40~~	
41	
42	
43	
44	

Blanks

31
32
33
34
35

Acclimatisingdays

1	H	1410	
2	H	1610	
3	H	1810	
4	H	1910	
5	B	1410	
6	B	1610	NRF
7	B	1810	
8	B	1910	
9	C	1410	
10	C	1610	
11	C	1810	
12	C	1910	
13	B	1810	Free
14	B	1810	
15	C	1810	Free
16	C	1810	
17	B	1810	A
18	B	1810	Glu
19	B	1810	S
20	B	1810	G
21	C	1810	A
22	C	1810	Glu
23	C	1810	S
24	C	1810	G
25	No long run		
26	No sprint		

27	H	1410	
28	H	1610	
29	H	1810	
30	H	1910	
31	B	1410	
32	B	1610	
33	B	1810	
34	B	1910	
35	C	1410	
36	C	1610	
37	C	1810	
38	C	1910	
39	Standards CAMP.		
40			
41			
42			
43			
44			

N° 4 tubes

0.1 ml 16 mM ATP → ATP (25 µl/tube)

0.1 ml 36 mM $MgCl_2$

0.1 ml 73 mM Theophylline

0.1 ml 1% Albumin

0.1 ml 1.8 mM $MgSO_4$

0.1 ml Tris HCl 7.4 + 7.4

0.1 ml Gly-Gly 3.6 mM

0.1 ml NaCl 16 mM.

Dissolve tubes in 4 ml Gly-Gly 4 × 12 ml Tris 1 M + 3.18 NaCl

ATP

16 mM .551 mg 1 ml 1 mM.

.551 mg .1 ml 10 mM

.883 mg .1 ml 16 mM

3.3 mg . 4 ml 16 mM.

36 mM $MgCl_2$

.203 × 141.4 = 2.9 ml

73 mM Theophylline .180 × 29.2 5.25 : 4 = 5.25

Albumin 4 mg → .4 ml

1.8 mM $MgSO_4$ .246 × 7.2 × 1/10 = .18 mg.

3.6 mM Gly Gly .132 × 144 × 1/10 = .19 mg

16 mM NaCl .092 × 58.4 = 0.2688 mg.

22/10/24 Trial Adenylate kinase

Incubate 15' 37° — brief 10' - Centrifuge

TO supt. Add 1:15 $Ba(OH)_2$ + 0.5 0.1 M.

Centrifuge.
1 ml O Column
0.5 ml dried Cond.
Take pale bands

0.08 ml "K ATP 10 4 ml
4 ml

1 H 1610 15'
2 B 1610 15' } 15'
3 C 1610 15'

.6 ml each.

+0.2 ml conc.

4 H 1610 - 0'
5 B 1610 - 0' } 0'

22/10/34

Vial 5.4025

Vial + $EuCl_3$: 5.4109 } 8.3

Stock

8.3 mg in 3 ml. ≈ 10^{-2} M

·257 mg in 1 ml. 1 m.M. 10^{-3} M

10^{-2} M

European charcoal ppt

0.3 ml of 8.3 mg in 3 ml. soln.
→ + 10 ml buffer.
↓ Mixed.

7.5 ml + 1.5 ml conc
(14/10 DIALYSED)

Neutral
2.5 ml + 0.5 ml buffer

Emission 495

Excitation Spectra 8×10 range. Emission 495.

I		Reading	II		3×10 Reading
	260 - 23		260 -	19.5	28
	270 - 22	44		17.5	27.5
	280 - 30	40		20.5	28
	290 40.5	49		23.5	30
	300 47	420 78 -		26	30
1	47	49		29.5	32.5
Blank	45.5	38		32	450 — 38.5
	47	44		33.5	32.5
	49 -	460 59		33	>100
	40.5	>100		31.5	
	48			30	
	47			29	
	45			28	

Blank Reads

38

Exciti 420

I / Emission Spectrum X10;8

460 -	77
470 -	62
	66
490	82
500	62
10	35
20	27
30	23
40	20
50	18
60	16
70	14
80	12
90	11
600	10
	9
10	8.5

Maximum

II 330 Exciti X10;3

400 -	71
10	60.5
20	54
30	48
40	43
50	39
60	36
70	35
80	31
90	28.5
500	26
10	22
20	19.5
30	17.5
40	15.5
50	14
60	13
70	11.5
80	10.5
90	9
600	8.5.

Maximum + Minimum

III DRUG X1;3

Excitation Spec. Exciti 350.

				Emission Spec	
260 -	6	370	61	370 > 100	
-	9		43.5	380 · 61	
80	14	390	32	85	
	18	400	24	103	
300	22	10	17	105	
10	32.5	20	15	> 105	
	46	30	13.5	420 < 410 -	All above 100
30	62.5	40	14	430 < 420	
	75	50	17	440 < 430.	
350 -	78 -	60	25	450 — 92	
	73	70	48	460 91	
			> 100	470 93	

Maximum 2 minima 0.05%

III

480 – 89
490 – 81
500 – 71
510 – 59.5
520 – 48
30 – 37.5
40 – 29
50 – 22.5
60 – 17
70 – 12.5
80 – 8
90 – 5

IV

Emission 495. X10 3. Excite 340.
Emiss. Spect

~~350 – 2~~
260 – 19
70 – 18
80 – 20.5
90 – 24
300 – 26
10 – 31
20 – 34
30 – 35
340 ← – 35.5
50 – 35.
60 – 34.5
70 – 34.5
80 – 33.5
90 – 32.5
400 – 32.5
10 – 36
20 – 36
30 – 36
40 – 39
50 – 48
60 – 65
70 – 100

400 – 99
80
69
61
56
51
47.5
45
40
37 (B)
33.5
31
28
24
22
19
17
15
14
13

plotted

(Blank + Dry 0.05 ml)

310

X1 j3.

Emission 470.

250 – 7
260 – 8
9.5
11.5
16.5
22.
31
42.
60.5
79
350. – 87
77
76
32.5
23
18
15
16
19
40
7100

Excite 350.

450 – 7 100
60 102
70 97
80 87.5
90 76
500 66
10 55
20 44.5
30 35.5
40 27

370 – 1X1

370 – 17
22.
39.
51.
410 – 59
58.5
53
47
39.5
34
30
480 26.

repeat End.

Dapoxyl

Young rat, adult, 3 starved animals, brown
—> pollen-like cells in Mohr.

cholesterol

Searcy et al.
Chem. Chim Acta
5 142 (1960)

Mol. Lipid in Engel J. Neurochem 3 157 (1958)

UV Spectra of Lipids in the Cent Pale

14 — 19 | 10 | 74

Preparation B C ch

Lipid

1 Ppt in chloroform.

Freeze dried

% Protein / Lipid wt

Phospholipid / total wt

Total Lipid / wt

} Ratios wts found at sometime.

Left over protein in cell
electrophoresis

Amino acid analysis Epsom salts

ATPhm

H, P_1, P_3, A, B, C

60 Lhm

Previous Prepn Dialysis

	Tube		
1	1S	—	B 1610
2	2S	—	C 1610
3	3S	—	B 1810
4	1B	—	C 1810
5	2B	—	B 1910
6	3D	—	C 1910

DIALYSED 24 hrs DDW

26/10/74 Procedure

Homogenate 3 gm in 60 ml (8.5% Sucrose in Tris Hcl. 10mM PH 7.4)

For Potter-Elvehjem – type Machine.
10 strokes 1000 rpm.

H. Homogenate Centrifuge 3500 rpm 3'

Supernatant I — 15000 rpm 15' pellet II
supt II

pellet II resuspend in 10-15 ml
layer over 10 ml 40% Sucr.

15000 30' pellet III Int I Supt III

Int I. suspend in 10 ml layer over.
35% 25% Sucr. 83000 in 45'

A B C

A | H Assay Na-K ATPase – / Balance A, B, resp by
B | pellet I. in all " Lactate . (D resp kamat.
C | supt I
susp. 5' / methodology all Lactate 10 Lactate
pellet III " in duplicate.
Int I A cholinesterase SD 1+ NAD 1+ duplicate
supt III .05 .05
HBC all small amount of resi 4 in Steroid.

Dehiat

26/10/74

Tissue

IYR 1. Less than 1/3 day old rats – 2. ⟹ 1.8 gms.

IIAR 2. Adult rat 1 ⟹ 1.4 gms

IIIM 3. Monkey Motor cortex ⟹ 3.2 gms

	Fraction	Volume ml.	Protein mg/ml.		
1	H IYR	50 ml			
2	H IIAR	55			
3	H III M	65			
4	P_1 IYR	10			
5	P_1 IIAR	15			
6	P_1 III M	25			
7	S_1 IYR	45			
8	S_1 IIAR	50			
9	S_1 III M	50			
10	P_2 IYR	20	~~10~~	S_2	40
11	P_2 IIAR	20	~~15~~	S_2	46 Blood mj: 6 ml
12	P_2 III M	20	~~25~~	S_2	50
13	P_3 IYR	} Very small amounts			
14	P_3 IIAR				
15	P_3 III M				
16	Int, IYR	10.5			

Fraction	Protein mg/ml	Volume ml	Total protein mg.
H I YR	2.7	50	135
H II AR	2.4	55	132
H III M	4.75	65	308.75
P_1 I YR	2.8	10	28
P_1 II AR	3.8	15	57
P_1 III M	5.5	25	137.5
S_1 I YR	2.6	45	117
S_1 II AR	1.9	50	95
S_1 III M	2.85	50	142.5
P_2 I YR	2.6	20	52
P_2 II AR	1.95	20	39
P_2 III M	3.0	20	60
P_3 I YR	Not Collected	Very small amts	
P_3 II AR			
P_3 III M	—		
Int$_1$ I YR	1.8	10.5	18.9
Int$_1$ II AR	1.65	12	19.8
Int$_1$ III M	2.45	14	34.3
S_2 I YR	1.0	40	40
S_2 II AR	0.95	46	43.7
S_2 III M	1.2	50	60
A I YR	0.6	10	6
A II AR	0.95	10	9.5
A III M	2.0	10	20
B I YR	0.65	7	4.55
B II AR	0.35	5	1.75
B III M	0.28	6	1.68
C I YR	0.19	4	0.76
C II AR	0.08	8	0.64
C III M	0.3	8	2.4

Volume Protein 3+7/ml

17 I int, II AR 12

18 I int, III M 14

19 S_3 I yR
20 S_3 II AR
21 S_3 III M
} 1 ml each ml 25 35 Mcx & Electrof.

22 A I yR 10 ml

23 A II AR 10 ml

24 A III M 10 ml

25 B I yR 7 C I yR 4

26 B II AR 5 C II AR 8

27 B III M 6 C III M 8

---

rat kt ATPase 0.1 ml
30' incubati 37°C

H

A cholinesterase

Recalculated

		µM/5m/ml
H I YR	52 µM/~~mg~~m/ml	61.66
H II AR	87 µM/5m/ml	84.42
H III M	22 µM/5m/ml	22.36
P_1 I YR	51 µM/5m/ml	62.12
P_1 II AR	66.6 µM/5m/ml	44.6
P_1 III M	19 µM/5m/ml	14.02
Int I YR	115 µM/5m/ml	84.6
Int II AR	171 µM/5m/ml	126.23
Int III M	62.2 µM/5m/ml	46.15
A I YR	~~79.75~~ MM/5m/ml (~~79~~25)	58.37
A II AR	101 µM/5m/ml	67.73
A III M	53.7 µM/5m/ml	39.506
B I YR	213 µM/5m/ml	156.7
B II AR	6~~4~~3.8 µM/5m/ml	473.3
B III M	516.9 µM/5m/ml	95.58
C I YR	241.7 µM/5m/ml	177.7
C II AR	617.5 µM/5m/ml	453.68
C III M	63.55 µM/5m/ml	46.7

26110174 — 0.1ml +2.3 +.1 +.1

A cholinesterase

	0	30	60	90	120	150	180
H I YR	33 .482	30.5	28	25.75	23.75	21.75	20 .699
H II AR	28.552	24.5	21.75	19	17	15	13.5 .870
H III MR	18 .745	17	16	15	14	13	12.25 .912
P$_1$ I YR	32 .485	30	28	26.25	24.5	23	21.75 .668
P$_1$ II AR	23.634	20.5	18.5	16.75	15	13.75	12.5 .904
P$_1$ III M	13 .886	12	11	10.25	9.5	8.75	8 1.007
S$_1$ I YR							
S$_1$ II AR							
S$_1$ III M							
P$_2$ I YR							
P$_2$ II AR							
P$_2$ III M							
S$_2$ I YR							
S$_2$ II AR							
S$_2$ III M							
Int, I YR	33 .482	30	28.25	25	22.5	20.5	19 .721
Int, II AR	34.469	30	26	22.5	20	18	16 .796
Int, III M	24 .620	22.5	21	19.5	18.5	17	16 .796
A I YR	38 .420	37.25	36.5	35.75	35	34	33.5 .475
A II AR	44.5 .352	42.5	41	39	37.5	36	34.5 .463
A III M	34 .469	32	30.5	29	27.75	26.5	25.5 .593

	0	30	60	90	120	150	180
B I YR	40.5 .392	38	36	33.5	31.5	30	28 .552
B II AR	40 .398	36.5	33	30	27	24.5	22 .558
B III M	43.5 .362	43	42	41.5	40.75	40	39.5 .404
C I YR	43 .367	42.25	41.5	40.5	39.75	39	38 .420
C II AR	44.5 .352	43.5	42.5	41.5	40.5	39.75	39 .409
C III M	40.5 .392	40	39.75	39.5	39	38.75	38.5 .414
P_2 Bleaching II AR							

Protein (2 + 5 + 0.5)

27/10/84

1 H I YR .05 .1	71.5 / 0.145	2.25	20 A II AR 0.1	89 / 0.05		
2 H II AR .05 .1	76 / 0.12	1.9	21 A III M 0.1	74.5 / 0.13		
3 H III M .05 .1	49 / 0.31	5.0	22 B I YR 0.1	88 / 0.055		
4 P_1 I YR .05 .1	67 / 0.175	2.8	23 B II AR 0.1	94.5 / 0.025		
5 P_1 II AR .05 .1	59 / 0.23	3.8	24 B III M 0.1	95 / 0.0225		
6 P_1 III M .05 .1	44.5 / 0.355	5.5	25 C I YR 0.1	97 / 0.0125		
7 Int. I YR .05 .1	78.5 / 0.105	1.6	26 C II AR 0.1	99 / 0.005		
8 Int. II AR .05 .1	79.5 / 0.1	1.65	27 C III M 0.1	95.5 / 0.02		
9 Int. III M .05 .1	71 / 0.15	2.45				
10 P_2 I YR .05 .1	70 / 0.155	2.6	Blank	100 / 0.0		
11 P_2 II AR .05 .1	75.5 / 0.1225	1.96	50 µg	93 / 0.0321		
12 P_2 III M .05 .1	64 / 0.195	3.0	100 µg	89.5 / 0.05		
13 S_1 I YR 0.1	70 / 0.155	2.6	150 µg	82.5 / 0.085		
14 S_1 II AR 0.1						
15 S_1 III M 0.1	78 / 0.11	1.9	A II - 0.95			
16 S_2 I YR 0.1	67.75 / 0.17	2.85	A III - 2.0			
17 S_2 II AR 0.1	87.5 / 0.0525	1.0	B I 0.84			
18 S_2 III M 0.1	88.5 / 0.0525	1.95	B II 0.38			
19 A I YR 0.1	84 / 0.075	1.2	B III 0.33			
	91.5 / 0.04	0.6	C I 0.19			
			C II 0.08			
			C III 0.3			

S1 nuclease

H I Y R	[redacted] 3.8 μM/mg prot/hr	3.8
H II A R	5.8 [redacted] μM/mg prot/hr	5.8 ✓
H III M	3.1 μM/mg prot/hr ✓	
P, I Y R	3.4 μM/mg/hr	
P, II A R	4.8 μM/mg/hr	
P, III M	2 μM/mg/hr	
I mt, I Y R	4.8 μM/mg/hr	
I mt, II A R	6 μM/mg/hr	
I mt, III M	5.1 μM/mg/hr	
A I Y R	5.5 μM/mg/hr	
A II A R	4.5 μM/mg/hr	
A III M	3.5 μM/mg/hr	
B I Y R	9 μM/mg/hr	
B II A R	14.3 μM/mg/hr	
B III M	9 μM/mg/hr	
C I Y R	13.1 μM/mg/hr	
C II A R	33.7 μM/mg/hr	
C III M	9 μM/mg/hr	

27/10/34

(1+ drops 2·5+1·5+1·2)

No solution in H₂O

5' methodan

30' 0·1 ml

H I YR	1	50 / 0·30	C I YR	31	86 / 0·065
	2	48 / 0·32		32	85 / 0·07
H II AR	3	38 / 0·42	C II AR	33	84 / 0·075
	4	38 / 0·42		34	84 / 0·075
H III M	5	36·5 / 0·44	C III M	35	85 / 0·07
	6	37 / 0·43		36	85 / 0·07
P, I YR	7	50·5 / 0·295	B Camle	37	100 / 0·0
	8	51 / 0·292	0·5 μM	38	47 / 0·35
P, II AR	9	29·25 / 0·53	1·0 μM	39	27·5 / 0·56
	10	29 / 0·535	1·5 μM	40	15 / 0·83
P, III M	11	46 / 0·34			
	12	44 / 0·355			
Int. I YR	13	57·5 / 0·24			
	14	55 / 0·26			
Int, II AR	15	53·5 / 0·2725			
	16	52 / 0·2825			
Int, III M	17	45·5 / 0·34			
	18	40 / 0·40			
A I YR	19	80·5 / 0·095			
	20	80 / 0·0975			
A II AR	21	73·25 / 0·1325			
	22	73 / 0·135			
A III M	23	60 / 0·22			
	24	56 / 0·25			
B I YR	25	70·5 / 0·15			
	26	69·5 / 0·16			
B II AR	27	72·5 / 0·14			
	28	73·5 / 0·135			
B III M	29	65 / 0·07			
	30	66 / 0·065			

(Uncle 3 ml

Wala not added

after developing colour)

27/10/54

Na-K ATPase 30' 0.1 ml 38°

(Baldwin AB Sum method D centrifuge) ½ l camal-

HI YR
1 Na 1: 31/0.505
2 K 2 28/0.555 → 1.23 MM/mg prot/hr
3 Na 3 30.5/0.515 (X) → 0.123
4 K 4 30/0.52

HII AR
1 Na 5 32.5/0.485 * 3.2
2 K 6 25/0.60
3 Na 7
4 K 8 30/0.52 2.22
25/0.60

HIII M
1 Na 9 13/0.83 ✓
2 K 10 10/1.0 2.39
3 Na 11 11.5/0.94 ✓
4 K 12 10.5/0.975 0.5

BI YR
1 Na 13 55/0.26 10.2
2 K 14 43.5/0.36 max
3 Na 15 56/0.235 14.79
4 K 16 41.5/0.38

BII AR
1 Na 17 69.25/0.16
2 K 18 50/0.30 26
3 Na 19 68.5/0.165
4 K 20 49.5/0.305 26

BIII M
1 Na 21 60/0.095
2 K 22 69/0.16 15.4
3 Na 23 79/0.10
4 K 24 67/0.175 17.8

S/A Blank - 25 100/0.0
0.5 mM Na 26 71/0.15
1 mM K 27 49/0.31
1.5 mM Na 28 35.5/0.415

(2+5+15')

27/10/74 Anolin Reikshanahi

1	HIYR	.1	63 / 0.20	3.00 mg/ml } 2.7
2		.2	46 / 0.34	2.44 mg/ml }
3	HIIAR	.1	68 / 0.17	2.5 mg/ml } 2.4
4		.2	49 / 0.31	2.3 mg/ml }
5	HIIIM	.1	45 / 0.345	5 mg/ml } 4.75
6		.2	28 / 0.55	4.5 mg/ml }
7	BIYR	.5	61 / 0.215	0.67 mg/ml } 0.65 mg/ml
8		1	41 / 0.39	0.62 mg/ml }
9	BIIAR	.5	73 / 0.13	0.36 mg/ml } 0.35
10		1	58 / 0.235	0.34 mg/ml }
11	BIIIM	.5	78 / 0.1075	0.3 mg/ml } 0.28
12		1	66.5 / 0.16	0.26 mg/ml }
13	B1610 DIAL	.1	90 / 0.0475	0.65 mg/ml
14	C1610 DIAL	.1	90.5 / 0.045	0.65 mg/ml
15	B1810 DIAL	.1	80 / 0.0975	1.35 mg/ml
16	C1810 DIAL	.1	83 / 0.08	1.13 mg/ml
17	B1910 DIAL	.1	66.25 / 0.16	2.3 mg/ml
18	C1910 DIAL	.1	93.5 / 0.03	0.43 mg/ml
M	Blank		100 / 0.0	
20	50 µg		92.5 / 0.035	
21	100 µg		87 / 0.06	
22	150 µg		81 / 0.09	
23	200 µg		71.5 / 0.145	

A B Balram Ashok · C. Kein(6)
D Kamal Ashok 3.9

7·20-2·50

28/10/74

Na-K-ATPase kepaar

30' 38°C 0.1ml

2nd

" H I YR { NA 1 32.25 / 0.49
K 2 29 / 0.54 → 1.23

" H II AR { NA 3 32 / 0.495
K 4 27.5 / 0.56 1.8

" H III M { NA 5 10 / 1.0
K 6 8 / 1.1 1.4

" B I YR { NA 7 53 / 0.275
K 8 41 / 0.385 11.22

" B II AR { NA 9 70.5 / 0.15
K 10 52 / 0.285 25

" B III M { NA 11 86 / 0.065
K 12 71.5 / 0.145 19.04

" A I YR { NA 13 73 / 0.135
K 14 63.5 / 0.195 6.94

" A II AR { NA 15 67 / 0.1725
K 16 46.5 / 0.335 11.4

" C III M { NA 17 82 / 0.085
K 18 79 / 0.1025 – 0.105 4.44

" B 1610 DIAL { NA 19 78 / 0.1075
K 20 82 / 0.085

" B 1810 DIAL { NA 21 66 / 0.18
K 22 66 / 0.18

" B 1910 DIAL { NA 23 76 / 0.15
K 24 82 / 0.085

Gene Case

Blank 25

Standards Same as previous Page

done on 27/10/74 0.300 = 1 mM

DSS, Verse
Lotrel 3 mL
Longest 0.1 mL

SDH

M Moles DCI reduced / mg protein / min

Formula
vol. x 0.1 mL
OD/min x
10.6 x mg protein/mL

H I YR	⟶	$1.509 \times 10^{-2} \times 2$
H II AR	⟶	$1.042 \times 10^{-2} \times 2$
H III M	⟶	$1.754 \times 10^{-2} \times 2$
P, I YR	⟶	$1.422 \times 10^{-2} \times 2$
P, II AR	⟶	$0.731 \times 10^{-2} \times 2$
P, III M	⟶	$1.886 \times 10^{-2} \times 2$
Int, I YR	⟶	$1.080 \times 10^{-2} \times 2$
Int, II AR	⟶	$0.9539 \times 10^{-2} \times 2$
Int, III M	⟶	$3.363 \times 10^{-2} \times 2$
A I YR		$3.394 \times 10^{-2} \times 2$
A II AR		$1.949 \times 10^{-2} \times 2$
A III M		$4.289 \times 10^{-2} \times 2$
B I YR		$0.2849 \times 10^{-2} \times 2$
B II AR		N D
B III M		1.654×10^{-3}
C I YR		N D
C II AR		N D
C III M		N D

Blank

Calculate +0.05 28/10/24

Codeine 2.85 +0.05 mg

600 nm

0.1 ml mcb

SDH

	0	30	60	90	120
H I YR	45 .347	42.25	49.75 .303	52	54.25
H II AR	39.5 .404	40.75	42 .377	43.5	45
H III M	33 .482	37	40.5 .392	44.25	48
P, I YR	43 .367	45.25	47.5 .324	50	53
P, II AR	36 .444	37	38.5 .414	40.25	42
P, III M	29 .538	33	37.5 .426	42.25	47.5
In, I YR	40 .398	41	42 .377	43.25	44.25
In, II AR	39.5 .404	40.25	41 .387	42	42.75
In, III M	35 .456	39	43 .367	47	51.5
A I YR	41 .387	42	43.25 .365	45	46.25
A II AR	41 .387	42	43 .367	44	45.25
A III M	38 .420	42.25	47 .328	52	57.5
B I YR	40.75 .389	40.75	41 .387	41.25	41.5
B II AR	43.25 .365	43.25	43 .367	42.75	42
B III M	41 .387	41.25	41.5 .382	41.75	42
C I YR	41.25	41.5	41.75	<42	42
C II AR	41.5	41.5	41.75	41.75	41.75
C III M	40.75	40.75	41.25	41.5	41.75
Blank + mj II AR	39 .404	40.5	42.5 .372	45	48

Unit : μmol NADH/mg prot/min.

H I = 110.1 millimols (in mitochondria/on mor prot)

H II = 119.4 millimols

H III = 99.04 millimols

P, I = 189.4 millimols

P, II = 78.12 millimols

P, III = 98.37 millimols

I_{nt}, I	257.4	millimols
I_{nt}, II	156.5	millimols
I_{nt}, III	246.8	millimols
A I	306.5	millimols
A II	152.6	millimols
A III	212.2	millimols
B I	76.17	millimols
B II	90.95	millimols
B III	88.41	millimols

I Largachil + Pyrene

II Erythro + Pyrene (Mixed late)

III Free Nuts

IV Trophs. of + 3rd Type

29/10/24 NADH Diaphorase 340 mm 0.1 ml

	0	15"	30"	45"	60"
H I	30.5" 0.516	34	37 0.432 0.168	39	40.5"
H II	21	23.5"	25.5" 0.593 0.162	27	27.5"
H III	14 0.698 0.854	17.	19 0.721 0.266	20	21
P I	19.5" 0.710	24	27.5" 0.560 0.300	30.5"	33.5"
P II	16.5" 0.783	18.5"	20 0.699 0.168	21.5"	23
P III	9.0 1.023	11.75	13.5" 0.840 0.306	14.5"	14.75"
Int I	12 0.921	14.25"	16.25" 0.790 0.262	19	21.5"
Int II	15 0.824	16.5"	17.75" 0.751 0.146	19	20.5"
Int III	10 1.000	12.25"	14.5" 0.839 0.342	17	19
A I	32 0.495	34.25"	36.5" 0.438 0.104	38.5"	40.5"
A II	30.5" 0.516	32	33.5 0.475" 0.082	34.75"	36
A III	22 0.658	26	29 0.536 0.240	31.25	33
B I	32.5" 0.489	33	33.5" 0.475" 0.025	34	34.75"
B II	35" 0.456	35.25"	35.75" 0.447 0.018	36	36.25
B III	28.75" .541	29	29.1" .534 0.044	29.5	29.75"
C I	27.5" .580	28	28.5" .545" 0.021	29	29.5
C II	13.75" .862	>13.25"	14 0.854 0.048	14	14.25
C III	21.75" .683	22	22.25 .853 0.020	22.5	22.75"

.19 C I - 279.2 units

.05 C II - 353.7 units

.3 C III - 117.9 units

29/10/20

I Acridine (400 µM)

0.5 ml + 9.5 ml Diluent

PH
① Acid
② Tris-buffer 7.4
③ Alkaline
④ ① + ② + Mab

II Tris 0.05 ml + 4.5 ml buffer. III Free mab : 1 in 5

IV Pyrene + eluant ① add dye ② & Pyrene as before

---

① Pyrene 1 mg + Triethyl 0.1 ml + 5 ml Tris 7.4

② Pyrene 1 mg + Lauryl 0.1 ml + 5 ml Tris 7.4

③ PH 14 (2.5 ml + 2 (NaOH 1N) + 2 Acr) Yellow free

④ PH 2 (2.5 ml + 2 HCl) + 2 Acr) Orange Red

Cuvette ① Free Acridine in PH 7.4 Tris 10 mM (dilution as ④)

Cuvette ② PH 14 Acridine —

Cuvette ③ PH 2 Acridine —

Cuvette ④ Free Acridine ① & Mab 1.5 ml to 5 ml

29/10/24

1 Accordi 0.5 mc + 9.5 mc Tris 10 mm 2.4 mm
(5 mc + 1.5 mc Impl). Debole corrente

Emilia MAX 525
(3x1 reg)
Excl

220	1.5
30	2.5
40	4.5
50	12
60	34
65	49.5
70	56
75	57
80	57.5
85	65
90	75.5
95	78.5
300	70.5
305	54
310	41.5
315	29
20	21
30	8
40	4
	6.5
	11.5
	15.5
	32
	49.5
4100	70
10	95.5 (3x1) 52 (2x1)
	70 (2x1)
	96.5 (2x1) 51.5 (1x1)
	73 (1x1) 26.5 (3x1/10)
4150	39 (3x1/10)

460	59 (3x1/10)
	75.5
475	76.5
480	79.5
485	84
490	85.5
491	80
500	68
505	51.5
510	36
515	22.5
520	15
530	4.5
540	1.5
550	1

3x1
Excl 295
Emilia

260	4.5
90	11.5
95	24.5
300	32.5
20	27
40	1
60	1
80	0.5
400	1
420	1
440	1
460	1.5
480	1.5
490	1
500	5
510	17.5
515	47
520	62
525	72
530	78
535	77.5
540	73
550	66
558	53
	40
	28
	17.5
	9
600	4.5

610	2
620	1
630	1.5
640	0
650	0

5 (3x1) 42.5 (1x1)
+10 (3x1/10)

(3x1/10)
Excl
490

470	0.5
80	2
90	8
500	20
05	35
10	51.5
15	68.5
20	77.5
25	83
30	82.5
35	77
540	69.5
545	62.5
550	54
60	40.5
70	28.5
	18
	8.5
600	4.5
	2.5
	1.5
	1
	1
650	0.75

Well 2 — Pit 141 — Acridine

0.2 ml Acridine + 2.5 ml buffer + 0.2 ml NaOH

(4 × 1/10 range)

Emission Max 540

Excit	
220	1
30	1
40	1.5
50	4.5
60	9
70	14.5
75	16
80	17
85	19.5
90	23
95	28.5
300	34
305	39.5
310	30
315	16
320	7
25	3.5
30	3
35	3.5
40	4.5
50	7.5
60	13
70	20
80	31
90	42
400	54
05	65
10	65
15	70
20	73.5
25	76

430	775
35	78.5
440	78.5
45	76.5
50	71.5
55	64
60	60
470	47
80	25
490	14
500	7
510	3
520	2

4 × 1/10

Excit	305
290	1
300	5
305	8
310	8.5
330	0.5
350	0.5
370	0.5
390	0.5
410	0.1
430	0.5
440	0.5
450	0.5
410	0.5
470	1
480	2.5
490	6
500	12.5
510	22
520	30
25	33
30	35
35	36
40	35
45	33.5
50	31.5
55	28.5
60	26
70	19
80	12.5
90	6.5

600	3.5
610	2
620	1
630	1

Excit	440
430	3
440	15
450	9
	1
	1.5
	4.5
	12
	26.5
510	46
520	63
25	68
30	71
35	72.5
40	71
45	66
550	60
60	46
	34
	21
	10
	5
610	2.5
620	1.5
630	1
640	0.5

Wells (3) Acetron PH.2.

(4 x 1) range

Emin Max. 5°65°

220	0
240	.5°
250	1.5°
60	3.5
70	7
80	10
85°	12
90	14.5°
95	16.5
300	16.5°
305	14.5
310	11
320	5°
330	4
340	7.5°
50	12
60	15°
65	16
70	15.5°
75	13
80	9
90	7.5°
4100	10
410	14
420	20
430	27
40	37
450	52
460	72
470	85. 84.5° 88.5
475	86.5
480	87.5
485	90
490	90.5°
495	86
500	76
510	52
520	34
530	24
5210	17
530	13.5
560	14
570	6
610	2.5°

(4x1)

Exuli 295

250	0
260	0
290	3
300	11
3106	
320	0
3100	
3600	(9x1)
70	0
80	.25
90	.5
400	.5
410	.5°
20	.5
30	.25
40	0
50	0
4600	
70	0
80	0
90	0
500	1
510	3
	5
	6.5
5440	8.5
	11
	15.5
	17
	15
	9
	4.5°
	2.5°

13.5	
19	
22.5	
24	
22.5	
19	
16	
13	
9.5	

620	1.5
630	0.5°
640	0
650	0

6x1 Exuli 365

16 (4x1) = 56 (6x1)

350	5
60	ovalio
70	ovalio
80	36
90	3
400	3
10	4.5°
20	5.5°
30	3.5°
40	2
50	1.5°
4601	.5°
	1.5
	2
	3
500	5.5°
10	11
	17.5°
	23.5°
	30
	40
560	51
565	58
570	57
575	54
580	47.5°

[541 (4x1)]

9x1 26

38	
48	
32	
19	
16	

590	29
600	15.5
10	9
20	4.5°
30	2.5°
40	1
50	0.5°

Archi (3)

Exhi 470 (4x1)

Campo 4mm

450	0
60	3.5
70	20.5
80	10.5
90	5
500	12
05	30.5
510	45
	58
	73
	79.5
	83
	84
	84
	84
	85
	86.5
	88
	89
	87
	80.5
580	70
90	41
600	22
10	12
20	7
30	4
40	2.5
50	1

Exhi 490 (4x1)

450	0
60	0
70	0
80	3
90	15.5
500	24
10	49.5
15	64.5
20	76
25	85
30	88
35	88
	87.5
	87
	87.5
	89.5
	91
	91
	84.5
	81
580	69.5
90	41
600	22
10	12
20	7
30	3.5
40	2
50	1

Cuelli (4) — Accrochage + Manœuvres (7-4 pm)

(3×1) range
Max Ouverture 530

Exercices (3×1 range)

220	0
230	0
240	0.25
250	1
260	3.5
270	4
280	5
290	5
300	5
310	4
320	1.5
330	1
340	0.5
350	2
360	3
400	4.5
410	6
420	6
	11.5
	16
	24
	36
	46.5
	50.5
	55
500	45

0	
1	
2.5	
8	
32	
37 → 38.5	
34	
35	
48	
46.5	
31	
16	
8	
5	
5.5	
9	
13.5	

510	32
520	69
525	84
530	53
540	5
550	0

Exculi 265 — 3×1

280	39
290	6
300	10
	17
	23.5
330	26.5
335	26.5
340	26
358	22
360	17
	12.5
	9
	6.5
	4.5
	3.5
	2.5
	1.5
	1
	1
	0.5

470	1.5
480	1
490	2
500	6
	16
	29
530	38
535	36
540	34.5
550	24
560	14.5
570	9.5
580	5.5
590	2.5
600	1
610	0.5
620	0
630	0

Exculi 295 — 3×1 range

310	49
320	48
325	49.5
330	49.5
335	50
340	48.5
345	46.5
350	43

360	33
370	24
380	17.5
390	12.5
400	9.5
410	7
420	5.5
430	4
440	3.5
450	2.5
460	2
470	1.5
480	1.5
490	4
500	11.5
510	28
520	45
530	51
540	49.5
550	35
560	26.5
	21
	24
590	31
595	35
600	29

$3 \times \frac{1}{10}$

495 Excite

480 – 6
56
495 – 91
500 – 88
510 – 42
– 47.5
25 52
30 52.5
35 50
40 45.15
50 36
60 26
70 19
11.5
5.15
2.15
1
0.15
0

I Lard + pyr
II Lard + pyr
III Free membr.
IV Triphro 05 + 3ml

(LARGACTIL + PYRENE)

I 465 Emission Excitation 360

4X1

250 – 3
60 – 3
70 – 4
80 – 5.5
– 5.5
5.5
7.5
10.5
66
29
54
360 – 80
72
50
35
26
19.5
17
– 19
51

390 – 53
400 – 43
26.5
37.5
46.5
60.
75
80
78
67.5
54
442
31.5
23
16
11
7.5
5
3

3X1

No. 2

FRAFL + PYRENE

No. 3

3X1

346

470 Emission

	Excitation 350.
250 — 2	370 — 49
1.5	39.5
6	53
11	62
15	63
19	60
21	58
38	60.5
53	64
67	67.5
350 — 70	— 70
59	67
40.5	60.5
24.5	53.5
16	45
12	37
9.5	29
9	22
10.5	16.5
16	12.5
>100	9
	5
	4

Emission 530.

250 — 1
-22
9.5
0
0
upto 350 — 0
0.5
upto 390. 0.5
450 — 0
460 — 0

3X10

8.5
8.5
upto 420 — 8.5
430 — 3.5

---

500 — 1.5
570 — 70
520 >100
530 >100
540 15
550 — 0

(Well numb)

NO3

Excitation 295

3 X 1

340 – 57
350 – 47
35
26
18.5
13.5
10
6.5
5.5
4
3.5
3.5
2.0
570 – 1.0
580 – 12
32
32.5
16
1
0

Free Mins

No. 4

Emission 465

3 X 1

250 – 17
18
19.5
20
24
29
33
39
69
made 84.5
360 — 95.5
92
79
69
62
58.5
65
82
>100.

Free Tryptophan

347

Excit 360

~~3 X 1~~

380 > 100
385 – 72
390 – 77
395 87
400 96
410 – 110 (~~2 X 1~~)
410 – 63 (2 X 1)
420 65.5 (")
430 64.5 (")
63 (")
~~#~~ 450 – 59.5
460 104 (")
470 98
480 – 87
75
65
57.5
49.5
40.5
32.
25.5
19.5
14
– –

Cirelli ① Free Accordine

Cirelli ② + Trifluo. olivine

cirelli ③ Bronchus + Mamb.

cirelli ④ Mandr + mamb + Trifluo. olivine

---

④ Bronchus + Mamb + Trifluoperazine olivine

3 x 1 range

Energia 530

exhaustion specks

220	1.5	705 (1x1)	26 (3x 1/10)
30	1.5	38 (3x 1/10)	
40	2	416 (3x 1/10)	
	2.5	50 (3x 1/10)	
	3	53.5 (3x 1/10)	
	5	41 (3x 1/10)	
	8	66.5 (3x 1/10)	
	10		
	10 25		
	12		
	15		
	22		
	31.5		
	39		
	44		
	45		
	44		
	47.5		
	56		
	69		
	88 (3x1)	47 (2x1)	
	63 (2x1)		
	90 (2x1)	48 (1x1)	

500

④ Comp 530 (3x 1/10)

280	.5
290	.75
300	.75
310	1
320	1
330	2
340	3
350	4
360	4.5
370	4.5
380	4
390	4.5
400	5.5
410	7
420	11.5
430	16.5
440	24.5
450	37
460	47
470	50
480	53
490	42
500	37
505	60
510	

Exacti 490

510	50
15	46
20	51
25	53.5
30	53
35	49
40	44.5
45	39.5
50	34
55	30
60	25
65	21.5
70	18
80	11
90	6.5
600	2
10	1
20	.5
30	0
40	0
50	0

Cuvette (4)

Excitation 365 — 3 x 1 nm

390	76
400	52
10	48
20	47.5
30	51
40	58
50	64
60	66
70	67
80	61.5
90	54.5
500	53
10	51.5
20	57
30	52
40	43
50	34
60	22
70	20
80	13
90	6.5
600	3
10	1.5
20	1
30	0.5
40	0
50	0

Excit 290 — 6 x 1 nm

300	8.5
310	0
320	0
330	0
340	1.5
350	3.5
360	11.5
370	28.4
380	47
390	68
400	84
410	89
420	90
430	82
440	86
450	83
460	81
470	78
480	66
490	58
500	57.5
510	65
520	69
530	63
540	50
550	39
560	29.5
570	21

560	13
590	7
600	2.5

Quelle (2/3)

[3 × 1/10] Accordement Mendy

Exuli max 525

Exuli

		(4×1)
250	0.5	13
60	1.5	33.5
70	3	57.5
80	3	60.5
90	4	80
300	4	76
10	2	45
20	1	24
30	0	9
40	0	5
50	0	7
60	0	12
70	1	21
80	1.5	35
90	2.5	53
400	3.5	76
10	5	100
20	7	
30	10.5	
40	15	
50	23	
60	35	
70	44.5	
80	47	
90	50.5	
500	40	
10	20	
20	8.5	
30	2	
40	0	

Exuli 490

Compensi (3 × 1/10)

490	6
510	14
520	32
530	47
540	50
550	43
560	33
570	24.5
580	17
590	10.5
600	5
10	2
20	1
30	0
40	0
50	0

Exuli 290 [4×1] rayé

		(9×1)
300	22	49
310	2	50
20	1	
30	1	
40	.5	
50	0	
60	0.25	
70	0.5	
80	1	
90	1	
400	1.25	
10	1.25	
20	1	
30	.75	
40	.5	
50	.5	
60	.5	
70	.75	
80	1.5	
90	6	
500	19.5	
10	50	
20	73.5	
30	77.5	
40	65.5	
50	51	
60	37.5	
70	26	
80	17	
90	8.5	
600	3.5	
10	1	
20	0	

310	72
315	39
320	46
325	53
330	50
335	43
340	37
345	36
350	35
360	33
70	38
80	50
90	61
400	66
10	64
20	58
30	51
40	46
450	42
	42
470	48
460	76

Exuli 365

[6×1] rayé

(9×1)	390	4	90
	400	3	800
	10	4	10
	20	6	20
	30	5	30
	40	3.5	40
	50	4	50
	60	4	60
	70	4.5	70
	80	6	80
			90
			600
			10
			20

10	
21	
41.5	
56	
57.5	
48	
37	
28	
20	
12.5	
6	
2	
1	
0	

Cuvette ② · Blank Accendue + Tryflus 104

Emission 530 (3×1/10 range)

		4×1
250	0	4
60	0	5
70	0.25	8
80	0.5	16
90	1	21.5
300	1	22
10	1	25.5
20	1.5	35
30	2	49.5
40	3.5	67
50	4	80
60	5	
70	5	
80	5	
90	6	
400	7	
10	9	
20	12	
30	16	
40	23.5	
50	35	
60	51	
70	66	
80	68	
90	72	
500	56	
10	54	
20	>100	

Exali 490 (3×1/10 range)

510	48
15	55
20	66
25	71
30	71.5
35	67
40	61.5
50	44
60	34
70	25
80	15
90	7.5
600	3.5
10	1.5
20	0.5

460	44
470	34
	33
	28
	27
	30
	31.5
	29
	23
550	18
	13.5
	8.5
	6
590	2.5

Exali 290 (6×1)

300	21.5
10	1.5
20	0
30	0
40	2
50	6
60	14
70	28
80	50
90	73
400	92
10	97
20	94
30	86
40	76
50	65
60	58
70	53
80	45
90	40
500	42
10	55
20	65
30	64
40	52
50	40
60	30
70	22
80	14
90	7
600	3
10	1
20	0

Exali 365 (4×1 range)

390	90	50
95	85	50.5
400	92	57
405	97	63
410	102	67
415		69
420		69.5
425		68.5
430		67
		64
		61
		56
450		51

29/10/24

avelli ① Accredine Free

(3×1/10) mg

Emin 530

		(4×1)	
250	0		255 10.5
60	2	13	255 20
70	3	48	260 45
80	3	60	265 61
90	4	57	270 61
300	4		275 57
10	2.5		280 57.5
20	1.5		285 64
30	1		290 75
40	1.5		295 65
50	.5		300 78
60	.5		305 65
70	1		310 52
80	1.5		315 40.5
90	2		320 32.5
400	3.5		
10	5		
20	6.5		
30	9.5		
40	14		
50	21.5		
60	33		
70	43.5		
80	47		
90	52		
500	43		
10	27		
20	50		
30	57		

(6×1) mg

6/1 Exuli 365

390	>100
395	71
400	55
405	47.5
410	42
415	40
420	38
425	36
430	33.5
435	32
440	31
445	30.5
450	29.5
460	27
70	25
80	23
90	23
500	29.5
10	47
20	63
30	68
40	59.5
50	48
60	38
70	28
80	19
90	10.5
600	6.5
10	4.5
20	3.5

3×1/10

Exuli 490

500	55
505	34
510	32
515	41
520	46
525	51
530	51.5
535	41
540	45
550	36
60	27
70	19
80	11.5
90	6
600	2.5
10	1
20	0.5

Exuli 290

(4×1) mg

305	>100
310	>100
15	62
20	66
25	71
30	74
35	74.5
40	72
45	68
350	63
60	49
70	42
80	36
90	29
400	22
10	15
20	11
30	8
40	6.5
50	5
60	4
70	3.5
80	3.5
90	7
500	18
10	46
20	48
30	75
40	66
50	52
60	41
70	43
80	56.5
90	41.5

600	15
610	3
620	1

11/III/74 - 12/III/74

Cholesterol estimation

		Coleman	Ether	Ichthl.
1	B 1610 DIAL	19 / 0.72	10 ml	194
2	B 1810 DIAL	8 / 1.1	10 ml	244
3	B 1910 DIAL	2.5 / 1.6	10 ml	310
(4.	C 1610 DIAL	51.5 / 0.29	4 ml)	97)
5.	C 1810 DIAL	3.5 / 1.45	10 ml.	298
Blank	—	100 / 0.0		0
Std 1 } 0.5 mg		57 / 0.24 21		80
Std 2 } cholesterol		54 / 0.27		85

Procedure

Mince fish piece (cholesterol ≃ 500 µg). Put 0.5 ml 40% NaOH + 10 ml ethanol - keep in boiling water bath for 15' (Saponification)

Petrol extraction twice.

Pool extracts, wash twice with final petrol layer with 80% Alcohol.

Dry over boiling water bath, clean petrol.

add 5 ml chloroform + 2 ml Reagent

Read colour after 15' upto 30' at 600 nm

Reagent : Acetic anhydride : Conc Sulphuric acid

4 : 1

Caution: do not allow to heat

add slowly with ice

Phospholipid estimation — Extraction Bligh & Dyer.

0.8 ml Suspension ⟹ 3 ml M:C 2:1 (Add impurities) ⟹ 2 ml $CHCl_3$

0.1 ml / sample.

Digest 170° perchloric acid 60%. Add reagents. Read in colorimeter (Klett-Summerson) 660 nm

60 mm	column
1	76 / 0.12
1'	71 / 0.15
2	83 / 0.08
2'	72 / 0.14
3	71 / 0.15
3'	73 / 0.135
4	84 / 0.075
4'	75 / 0.125
5	89 / 0.05
5'	67 / 0.175
6	65 / 0.19
6'	69 / 0.165
Blank	100 / 0.0
Std 1	2 µg. $P_i O_4$ 86 / 0.061
Std 2	2 µg. $P_i O_4$ 86 / 0.061

Boat effect. air washing

Repeated

14/11/74

Phospholipid Estimation

0.8 ml mix ⇒ 2 ml $CHCl_3$.

Dissolved 0.25 ml in 1 ml perchloric acid 60°.

0.5 ml molybdate + 0.2 ml ANSA
⇒ make upto 10 ml. Std 2 µg phosphorus in 1 ml.
620 nm.

		Colemans	Phosphorus µg	phospholipid µg/ml	Optical density µg/ml	µg phospholipid / µg phosphorus (Kates)
B1610	1 0.25	93.5 /0.03	1.32			
	1' 0.25	93.5 /0.03	1.32	330	0.65 ✓	520
B1810	2 0.25	89 /0.0525	2.32	580	1.35 ✓	428
	2' 0.25	89/0.05				
B1910	3 0.25	86.5/0.065	2.88	720	2.3 ✓	314
	3' 0.25	85/0.07				
C1610	4 0.25	95/0.025	1.1	274	0.65 ✓	414
	4' 0.25	94.5/0.025				
C1810	5 0.25	89/0.0525	2.5	624	1.13 ✓	556
	5' 0.25	87.5/0.0575				
C1910	6 0.25	93.5/0.03	1.32	330	0.43 ✓	766
	6' 0.25	93/0.035				
	Blank	100/0.0				
	Std 1 2µg	90/0.045				
	Std 2 2µg	90.5/0.0425				

1	0.25ml	30				
1'	B1610	31	1.348	337	0.65	518.45
2	B1610	50	2.217	554	1.35	410.39
2'		52				
3	B1910	59	2.677	669	2.3	~~290.87~~
3'		64				290.87
4	C1610	33	1.369	342	0.65	526.14
4'		30				
5	C1810	51	2.304	576	1.13	509.91
5'		55				
6	C1910	33	1.434	358.5	0.43	834.06
6'		33				

Blank 0

Std 1 2µl 46

Std 2 2µl 46

(30' 0.1 ml) (cyclohexamide)

	DIFF (a)		MMOL (axb)	Enzyme /mg mol/hr.	
H I	− 0.025* + 0.025	} 0.025	0.083	0.1752 mμ	
H II	0.05 0.05	} 0.05	0.166	0.2975 mμ	
H III	0.2* 0.05	} 0.05	0.166	0.2927 mμ	
B I	0.065 0.0725	} 0.0688	0.229	6.869 mμ	
B II	0.165 0.17	} 0.1675	0.558	9.305 mμ	
B III	0.19 0.215	} 0.2025	0.675	10.90 mμ	
B T27	0.13	} 0.13	0.433	8.66 mμ	

ATP Conc = 3 mg/ml (Kaul-prasanna)
(Contact D. Balan AB.)

27 obase 100/0.0
18 1 μM
24 2 μM
30 3 μM

15/11/74

		Wt	Homogenate volume
I	Monkey Cortex (CT 4/10/74 Kapthurgan)	= 3.3 gm	40 ml
II	Adult Rats - 2 -	3.4 gm	42 ml
III	Adult Rats 2 -	3.2 gm	44 ml

16/11/74 ultra centrifuge 40 K/s 6 hours

A's - 2 ml

B's - 5 ml

C's - 6 ml

7.35 - 8.05 0.1 ml 37°C

Na-K ATPase

HI	1 Na	~~7.5 / 1.15~~	
	2 K	4.5 / 1.35	
	3 Na	5 / 1.3	
HII	4 K	4.5 / 1.35	
	5 Na	5 / 1.3	
	6 K	4.5 / 1.35	
	7 Na	4.5 / 1.35	
	8 K	4 / 1.4	
HIII	9 Na	8 / 1.1	
	10 K	8.5 / 1.075	
	11 Na	8.5 / 1.075	
	12 K	8 / 1.1	
BI	13 Na	85 / 0.07	
	14 K	73.5 / 0.135	
	15 Na	85.5 / 0.07	
	16 K	72 / 0.1425	
	17 Na		
BII	18 K	50.5 / 0.295	
	19 Na	35 / 0.46	
	20 K		
	21 Na	47 / 0.33	
BIII	22 K	31.5 / 0.50	
	23 Na	49 / 0.31	
	24 K	31.5 / 0.50	
	25		
BT	26		

BIII	23	47 / 0.33
	24	28.5 / 0.5745
	25	59 / 0.23
BT	26	44 / 0.36

Blank		100 / 0.0
1 μM		47 / 0.33
2 μM		25 / 0.60
3 μM		12 / 0.925

16/III/34 Protein

.02 HI	1	.02	74.5 / 0.13	.1425		9.5 mg/ml
.02	2	.02	70 / 0.155			
.02 HII	3	.02	72.5 / 0.14	.1675		11.16 mg/ml
.02	4	.02	65.25 / 0.195			
.02 HIII	5	.02	69.5 / 0.16	.17		11.333 mg/ml
.02	6	.02	66 / 0.18			
.1 BI	7	.1	91.5 / 0.04	.05		0.6667 mg/ml
.2	8	.2	79 / 0.10	.1		
.1 BII	9	.1	82.5 / 0.085	.19		1.199 mg/ml
.2	10	.2	65 / 0.1875	.18		
.1 BIII	11	.1	80.5 / 0.095	.195		1.283 mg/ml
.2	12	.2	64.5 / 0.19			
AI	13	.04	79 / 0.10			3.33 mg/ml
AII	14	.04	76 / 0.12			4 mg/ml
AIII	15	.04	81 / 0.0925			3.08 mg/ml
CI	16	.1	79.5 / 0.10		→	1.33 mg/ml
CII	17	.1	74 / 0.13		→	1.733
CIII	18	.1	67.5 / 0.17		→	2.267
	23	Blank			=	100/0.0
	24	1 mm (100 µ)			=	86/0.065
	25	2 mm (200 µ)			=	71/0.15
Int I	4	.04	7.00	62 / 0.21		
Int II	4	.04	8.33	56 / 0.25		
Int III	4	.04	7.00	62 / 0.21		
12 BT	0.1		1 mg/ml	84 / 0.075		

A cholinesterase — μMol/g mol/mt.

HI	10.0062 ✓	limits μMol/Ln/mt
HII	28.158	"
HIII	28.119	"
BI	22.942 ✓	
BII	132.31	
BIII	118.19	
AI	22.589 -	
AII	49.842 ✓	
AIII	68.454 -	
CI	10.699 -	
CII	73.536	
CIII	49.453 -	
Int	19.570 -	

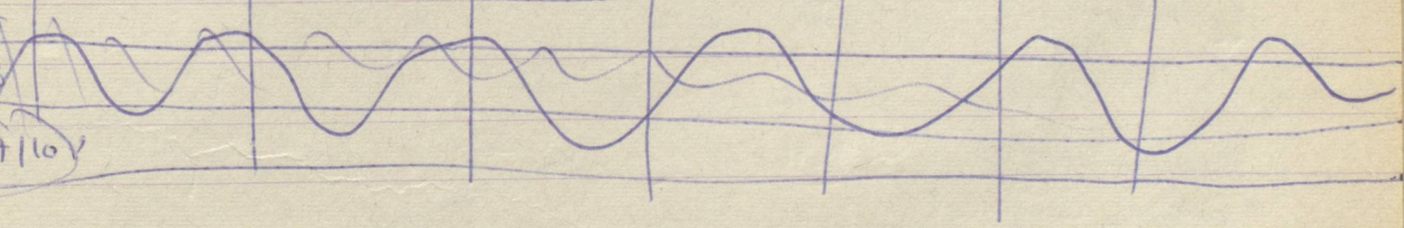
16-11-74

17/11/74

0·1 + 1·1 + 1·1 + 2·3

Acetyl cholinesterase

	0	30	60	90	120	150	180
H I	12 0.921	11.25	10.75	10	9.5	9	8.5 1.071
H II	8.5 1.071	7	5.25	4	3.5	3	2.75–3.0 1.568
H III	7 1.155	5.25	4 (1.14)	3.25	2.75	2.5	2.25 (1.775) 1.655
B I ⓐ	30 0.523	29.5	29.5	29	28.75	28.5	28.25 .578
B I ⓑ	24 0.820	23.75	23.5	23.25	23.25	23	22.75 .643
B II ⓐ	27 .569	24	22	20	18	16.5	15 .824
B II ⓑ	22 .658	20	18	16	14.5	13.25	12 .921
B III ⓐ	26.5 .571	24	22	20	18.5	17	15.5 .810
B III ⓑ	25.5 .593	23	21	19.25	17.75	16.25	15 .824
A I	20 .699	19.25	18.25	17.5	16.75	16	15.25 .817
A II	19.5 .710	17	15	13.25	11.5	10.5	9.5 1.023
A III	20.5 .688	18	15.5	13.5	12	10.75	9.5 1.023
C I	20 .699	20	19.75	19.5	19.5	19.25	19 0.721
C II	19 .721	17.5	16.25	15	14	13	12 0.921
C III	18 .745	16.75	15.75	14.75	13.75	13	12 0.921
I mH1	9 1.046	>8.5	7.75	7	6.5	6	5.5 1.261
I mH2							
I mH3							
BT 27/10							



18 III/74

NADH dehydrogenase

0.1 ml. 340 nm

	0	15	30	45	60
H I	~~27.75~~	~~27.75~~	~~27.75~~	~~27.75~~	~~27.75~~
	12	12.25	12.5	12.75	13
	12.5	12.75	13	13	13.25
H II	10.5	11	11.25	11.5	12
H III	9.5	10.25	10.75	11	11.5
B I	27.75	27.75	27.75	27.75	27.75
B II	27.5	27.5	27.5	27.5	27.5
B III	27	27	27	27	27
A I	17	17.5	—	—	—
A II	32	33	—	—	—
A III	—	—	—	—	—

.132.1 × 7.2
.02642
.9247
.95112

.042 × 32
.0084
.126
1.344

.5512 × 32
11024
16536
176384

Mgcl
.203.31 × 72
40662
142217
14.626832

.1802 × 146
10812
7208
1802
26.3092

.2465 × 3.6
14790
7395
.887400

120.

		Weights Enzymes Pmoles/mg protein	Hr
A I	3031.5		
A II	6279.4		
A III	6916.6		
B I	6520. (1304)		
B II	11186.0		
B III	14945.		

For 20 ml. Cocktail

			Weight
ATP	32 μmoles	ATP	17.6384
$MgCl_2$	72 μmoles	$MgCl_2$	14.62832
Theo	146 μmoles	Theo	26.3092 mg
Albm	20 mg/ml	Albm	20 mg
$MgSO_4$	7.2 μmoles	$MgSO_4$	0.95112 mg
$MgSO_4$	3.6 μmoles	$MgSO_4$	0.8674 mg
NaF	32 μmoles	NaF	1.344 mg
Tris 1M	→ 2 / 3.33 = 0.6 ml		

17.4 ml H_2O

.9 + 0.1 ml {mg dilution}
Cocktail

19/11/74

Adenylate Cyclase Assay

Pmoles cAMP

1 ml. mixture used

	Blank Control	Incubation 15' I	Incubation 15' II	Total cAMP in incubation mixture
H I 0.02	.02 146 Direct Boiling 1	0.02 132 7 15' incubation	0.02 192 8	162-18 144
H II 0.02	.02 96 Direct boiled 2 26	0.02 330 9 15' incubation	0.02 390 10	350.4
H III 0.02	.02 Direct Boiled 3	0.02 450 11 * (less additional incubation)	0.02 390 15' incubation 12	392
B I 0.1	0.1 96 4 Direct Boiled 13	0.1 45 15' incubation 14	0.1 66	48.9
B II 0.1	0.1 9.7 Direct 5 15	0.1 330 15' incubation 16	0.1 360	335.3
B III 0.1	0.1 Direct 6 6.6 17	0.1 450 15' incubation 18	0.1 510	473.4

Incubation mixture

1.6 mM ATP

3.6 mM $MgCl_2$

7.3 mM Theophylline

0.1% Albumin

0.36 mM Gly_2-Gly_2

0.18 mM $MgSO_4$

30 mM Tris HCl PH 7.4

NaF 1.6 mM

+ Enzyme + H_2O to make 1 ml.

15' 37°C

Boiling and Termination

Terminated by Boiling Extract.

338 µg/ml : 1 µM

1336 µg : 1 µM

5×10^{-6}

5 µM

6 µg/ml ≈ 20 µM

2.15×10^{-5} M.

21/11/74

(Mems 8 ml + 18 ml) Protein

Dried

1. Memb 1 ml — 75.5 / 0.12 = 150 µg
2. Memb 1 ml — > 75.5 / 0.12
3. Mem — 100 / 0.0
4. .2 (500 µg/ml) = 100 µg — 83 / 0.08
5. .4 (500 µg/ml) = 200 µg — 70 / 0.155

20/11/74

26/10/74 BI AR, BII AR } Combined Diagram 16 hrs
+ 15 11 BII AR BIII AR }

" BIII M + 15 11 BI M " = 330 μM/ml

Pyrene 202

·01 ml 6 3 ml. Should be 0.5 μM. P I
2 μM. P II
6 μM P III
12 μM. P IV

Conc Pyrene

P_I = 0·5 × 300 μM = 150 μM. Dil P_{II} × 1/4

P_{II} = 2 × 300 μM = 600 μM Dilute P III × 1/3

P_{III} = 6 × 300 μM = 1·8 mM. Dilute P IV 1/2

P_{IV} = 12 × 300 = 3.6 mM. ∴ ·202 × 3·6 = 5/ml

72
72
·727 2

7.272 in 10 ml

3.636 in 5 ml

(Ethyl alcohol.)

P_{IV} = 3.64 mg in 5 ml. Alcohol (Calculation)

4.8669
4.8711
4.2

Actually taken 4.2 mg in 5 ml.

Mems (6 ml Mems + 18 ml buffer.)

Mems ~~2 ml + 4 ml~~ buffer. ⇒ 6 ml pensel. 4
= 150 μg/ml.

BSA. 100 μg/ml in buffer ⇒ ✓

Ovalbumin. 100 μg/ml in buffer ⇒ ✓

I Membrane

A : Mem + Pyr I
B = Mem + Pyr II
C = Mem + Pyr III
D = Mem + Pyr IV

8 ml Rab B Dial
+18 ml Buffer
" 6 ml + .02 ml pyr (3 × 1/10 range all).

Wave Length	A	3 × 1/10	B	3 × 1/10	C	3 × 1/10	D	3 × 1/10
					Emission 390			
250	NO peak ~~not~~ sensible	1	250	2	3		3.5	
55							5	
60		1	55	2.5	4			
65							7	
70		1	60	3.5	5.5			
75		1	65	5.5	8.5		10.5	
80		1.5	70	8	12		15	
85		1.5	75	9	13		16	
90		2	80	7.5	11		13.5	
95		2	85	5.5	8		10	
300		2	90	5	7.5		9.5	
305		1.5	95	5	8		10.5	
310		1	300	6	10		14	
315		1	05	8	14		20	
320		1	10	11	19.5		27	
325		1	15	16.5	29.5		40.5	
330		1	20	20.5	37		49.5	
335		1	25	23	41.5		55.5	
340		1	30	26	50		66.5	
345		1	35	35	60.5		79.5	
350		1.25	40	30	51.5		66.5	
355		1.25	45	15	26		36.5	
360		1.25	51	5.5	9		14	
		1.5	55	2.5	4		7	
		1.5	60	2.5	3.5 ~~3.75~~		5.5	
		2	65	3	3.5		5	
		9	70	9.5	10		12	
		74	75	76	78		84	

Anthracene + Pyrene (3×1/10) all

wavelength	A	B	C	D
	Excitation 340			
355	5·8	56·5	55 58·5	5·6
60	11·5	13	60 14·5	14·5
65	2·5	7	65 10·5	12·5
70	1·5	13	70 22	25·5
75	1·25	19·5	75 33·5	41·5
80				
85	1·25	23·5	80 40·5	50·5
90	1	25·5	85 44	57
95	1	26·5	90 46	61·5
400	1	25	95 44	58
05				
10	1	21·5	400 38	50
15			405	
20	1	17	30	40
25				
30	1	14	10 25	34·5
35	1·75	12	15 22	30·5
40				
45	1	10	20 18·5	27
50	1	8	25 15	23·5
55				
60	0·5	6·5	30 13	21·5
65	1	5	35 11·5	21
70				
75	1	5	40 11·5	22
80	0·5	4·5	45 12	24·5
85	0·5	4·25	50 12	26
90				
95	0·5	4	55 12·5	25·5
500	0·5	4	60 13	29·5
05				
10	0·5	4	65 13·5	32
15				
80	0·5	4	70 13·5	31·5
25	0·5	4	75 13·5	32·5
30				
35	0·5	3·5	80 13	29·5
40	0·5	3·5	85 12	29
45	0·5	3	90 11	26
	0·5	2·75	95 10	24·5
		2·5	500 9	21·5

500

(3 × 1/10)

A	B	C	D	
0·5⁻	2·25⁻	8	19·5⁻	505⁻
0·5	2	7	16	570
0·5	<2·	6	13·5⁻	
0·25⁻	1·5⁻	5	11·5⁻	
0·25	1·25⁻	>4·	10	
0·5	1	3·5⁻	8	
0·5⁻	1	3	7	535⁻

---

yield, ~~very~~ half — BSA — 3 × 1/10 range

100 pg/ml Pyr

Gonadotropin 390.

6 ml + 0.02 Pyr

(2·5 ml to uterus.)

Pyrrolytic	Exercise Specific A BSA + Pyr I	B BSA + Pyr II	C BSA + Pyr III	D BSA + Pyr IV
2·20				
3·25⁻	0	1·5	2	2
0·25⁻	0·5	2	2·25	2·5
65				
260	0·5⁻	<3·5	>3·5⁻	>3·5⁻
265⁻	0·5	5·5⁻	5·5	5·5⁻
270	1	7	<8	7·5⁻
	1	7·5	8	8
	1	6	6·5	6·5
	1	4·5⁻	5	5
	1	4	4·5	5
	~~0·5~~ 1	4	5	5
	0·5⁻	5·5⁻	6·5⁻	6·5⁻
	0·5⁻	7·5⁻	8·5	9
	0·5	10·5⁻	12	12·5⁻
	0·5	16	18	18
	0·5⁻	19·5⁻	22	22
	0·5	22	25	24·5⁻
	0·5	26	31·5	31

(350)

BSA (3 × 1/10 mm)

Exakin spec

A	B	C	D	
<0.5	32	35	34.5	395 (335
0	20	22	22	340 (340
0.5	7	8.5	9	345 (345
0.5	2.5	3.5	5	
0.5	1.5	2.5	4	
0.5	1	2.25	4	
0.5	1	2.5	4.5	
0.5	1	5	10	
			7L1	
[illegible] 1	3.5	32		
2	9	91	≃240 mm	Stalli peak

Exali 335 3 × 1/10 mm

Constr spec

A	B	C	D
.5	1.5	5.5	14
0.5	1	2	3
0.25	3	3.5	3.5
0.5	8.5	9	9
0.5	20.5	22.5	22.5
0.5	29	32	31
0.5	30	33	32.5
1.5	31	33.5	32.5
0.5	32	35	34
0.5	30.5	33.5	33
0.5	25.5	28.5	28.5
0.5	19	21.5	22
0.5	15.5	18	19.5
0.5	13.5	16.5	18

	A	B	C	D
350	0.5	10.5	14	17
52				
60	0.5	8	11.5	15.5
	0.5	6	10	14.5
	0.25	4.5	8.5	14
	0.5	4	6	14.5
	0	3	8	15
50	0	2.5	7.5	15.5
55	0.5	2	7	15
60	0.5	1.5	7	15
65	0	1.5	6	14
70	0	1	6	14
415 475	0..	1	5.5	13

Cont'd next

Exclusion 335 — Lundin — BSA — 3x1 hr

A	B	C	D	?
0.5	1	5.5	12.5	480
0	1	5	11	85
0	1	4.5	10	90
0	0.5	3.5	8	90
0	<1	3	7.5	
0	>0.5	3	6.5	
0	0.5	2.5	5.5	510

Ovalbumin — 100 µg/ml — range?

A + Pyr I
B + Pyr II
C
D + Pyr IV

Exclusion: 390

Exclusion Lundin

315

?	A	B	C	D	A	B	C	D
25	0	1	1	1	0.5	9.5	11.5	12
55	<0.5	1	<1.5	1.5	0	10.5	13	13.5
60	<0.5	1.5	2	2	0	12.5	15	15.5
15	0.5	2.5	3	3	0	16	19.5	21
70					0	16	19.5	21
75	0.5	3	3.5	4	0.5	8.5	10.5	11.5
60	0.5	2.5	3	3.5	0.5	3.5	4.5	5
65	1	2	<2.5	2.5	0	1	2	2.5
80	1	>1.5	2	2.5				
95	1	2	2.5	2.5				
300	1	2.5	3	>3				
05	<1	3.5	4	4.5				
90	0.5	4.5	5.5	<6				
95								
3107	0.5	6.5	7.5	8				

Ovalbumin 3 × 1/10 mg. .02 Prot
0.6 ml
(100 µg/ml
protein)

Exp. 332.5 (Dilutions 7/10)

	A	B	C	D
350	0.5	0.5	2	3.5
60	0.5	2	2.5	2.5
70	0	14	17	17.5
80	0	17.5	21	21.5
90	0	17.5	21	22
400	0	14	17	18.5
10	0.5	8	10.5	11.5
20	0	5.5	8	9.5
30	0	3	5.5	7.5
40	0	1.5	4.5	7
50	0	1	4	7
60	0	0.5	3.5	6.5
70	0	0.5	3	6.5
480	0	0.5	2.5	5
490	0	<0.5	2	4.5
500	0	0	1.5	3

I^B — Mem b + Pyr III + Buff .01

II^B — Mem b + Pyr III + Dmy .01

III^B — BSA + Pyr III + Buff .01

IV^B — BSA + Pyr III + Dmy .01

I^A = Mem b + Pyr I + Buff .01 ⟵

II^A = Mem b + Pyr I + Larzacil .01

III^A = BSA + Pyr I + Buff .01

IV^A = BSA + Pyr I + ~~Buff~~ Dmy LARZACIL .01

Larzacil = Chlorpromazine Hydrochloride 25 mg/ml

340-390 $3 \times \frac{1}{10}(11.5) \equiv 6 \times \frac{1}{11}(76)$

PI : ·5 μM PII : 2 μM PIII : 6 μM PIV : 12 μM
final Conc in 3 ml of ·01 is 3 ml

3891

I = Memb 3ml · +·01 (150 mg/ml) PI
II = BSA = 3ml +·01 PI
III = Ovalbumin 3ml+·01 PI
IV = Alcohol 3ml + ·01 PIV

} As for the previous spectra

Excitation spectrum · Emission: 390

λ	I	II	III	IV
	3 x 1/10 range all			
250	1	0.5⁻	<0.5⁻	1
60	1.5	<1.5	1	2
70	3	3	>2.5	4
80	3	<2.5	1	2
90	2.5	<2	<1.5⁻	2.5⁻
300	<2.5⁻	2	2	7
10	4	4	3	13.5⁻
20	7	7	5⁻	20
30	9.5	10.5	8	27.5⁻
40	10	7	4	12
50	2	1	0.5⁻	2
60	<1.5⁻	0	0	1
70	15.5⁻	0.5⁻	0	0.5⁻
80	Off 100	3.5⁻	2.5⁻	1

λ	IA	IIA	IIIA	IVA
	(6 x 1/10 Range)			
360	17	19.5	6	12.5⁻
370	39.5	22	47.5⁻	39
360	65	24	63	49
390	75	21	65.5⁻	47
400	59.5	17	49.5⁻	37
10	40.5	14	31	25⁻
20	29	13	22	19
30	17	16.5⁻	11.5⁻	15.5⁻
40	12	19	7.5⁻	17
50	8.5⁻	25	4.5⁻	19
60	6.5⁻	24.5⁻	3	17
70	6	21.5⁻	2	16
80	5	19.5⁻	2	16
90	4	13	1.5⁻	12.5⁻
500	3.5⁻	10	1	11.5⁻

Emission spectra Excitation = 335

λ	I	II	III	IV
	3 x 1/10 range all			
350	30.5⁻	0.5⁻	0.5⁻	>0.5⁻
60	>2	1	1	<3
70	7	>8	8	18
80	11	11	8	26
90	>12	>11	>8	27.5⁻
400	<10	8.5⁻	6	20.5⁻
10	6.5⁻	5.5⁻	4	13.5⁻
20	4.5⁻	4	2.5⁻	9.5⁻
30	3	2	1.5⁻	5⁻
40	2	<1.5⁻	1	3
50	1.5⁻	1	0.5⁻	1.5⁻
60	1	0.5⁻	<0.5⁻	1
70	>1	0.5⁻	<0.5⁻	<1
80	1	<0.5⁻	0	0.5⁻
490	<1	>0	0	0

λ	IB	IIB	IIIB	IVB
	(3 x 1/10 range)			
360	5	3	2.5⁻	<2.5⁻
70	23.5⁻	4.5⁻	19	9.5⁻
80	45⁻	6	29.5⁻	14
90	54.5⁻	5.5⁻	31	14
400	44.5⁻	4.5⁻	24.5⁻	11.5⁻
10	29	3	16	8
20	21.5⁻	>2.5⁻	12.5⁻	6
30	14.5⁻	>2.5⁻	8.5⁻	5⁻
40	13	>3	7.5⁻	<4.5⁻
50	14	3.5⁻	7	5
60	14.5⁻	3.5⁻	6	4
70	16.5⁻	3	5.5⁻	<5
80	15.5⁻	2.5⁻	5	>3
90	13	2	4	3
500	10	1.5⁻	3	2
510	8	1	2	1.5⁻
520	5.5⁻	<1	1.5⁻	1

3 x 1/10 kx

I^c Oxalbm + Pyr I + Bmph

II^c " + " + Dmg.

III^c " + Pyr III + Bmph

IV^c " + " + Dmg.

I D Mlmb + Pyr II + 0.2 GABA

II D Mmb + Pyr II + 0.2 Asp

III D Mmb + Pyr IV + 0.2 Bmph

IV D Mmb + Pyr IV + 0.2 Mg^{++}

$20.5\ (3\times\frac{1}{10}) = 70\ (5\times\frac{1}{10})$
332–390

$5\times\frac{1}{10}$ rays Exch: 332.5

Exch 340

$3\times\frac{1}{10}$ rays

λ	I C	II C	III C	IV C	I D	II D	III D	IV D	λ
350	1	2	4.5	3	—	—	—	—	—
360	5	5.5	9	8.5	9	7.5	10.5	11.5	360
370	32	15	59	34	12	11.5	26.5	27.5	70
380	37	17	68.5	39	20.5	19.5	44.5	47	80
390	38.5	16.5	71	40	24	22.5	53	56.5	90
400	28.5	12.5	53.5	30	18.5	17	41	44	400
10	17	8.5	34	19.5	12.5	12	28	30.5	10
20	12	6.5	26	15.5	9.5	8.5	23	24.5	20
30	6	6	16	11	5.5	5.5	18.5	20	30
40	3.5	7	13.5	11.5	4.5	4.5	20.5	21	40
50	2	8.5	11.5	14	4	4	26	26	50
60	1	9	11	19.5?	3.5	>3.5	30	30	60
70	1	8	9.5	12	3	>3.5	32.5	32.5	70
80	0.5	7.5	7.5	10	3	<3.5	31	32	80
90	0.5	6.5	5.5	8	2.5	2.5	27	27	490
500	0.5	5.5	4.5	7	—	—	—	—	

Buf A: 62.5 mM NaCl + 1.25 mM $MgSO_4$
B = 50 mM KCl + above
Mix C = 5 mM ATP
D = 3.12×10^{-4} EDTA 8.6 pH
7 ml .0625 M
Enzyme 0.1 ml.

Na – K ATPase

	diff.	μMoles/ml/hr	Protein	μMoles/hr/mg prot
H I	1.06 1.025	~~Brey~~ 5.0	10.5	0.4762
H II	−1.05 × +0.05 ✓	5.883	10	0.583
H III	+0.27 × −1.045 ×	—	6.54	ans
PND I	0.0125	1.471	5	0.2941
PND II	0.1	11.766	4.8	2.451 *
PND III	0.06	7.06	6	1.177
LS I	0.195	22.95 ~~7.06~~	4.6 (?)	4.999
LS II	0.035	4.118 ~~22.95~~	5.3	0.7769
LS III	0.13	15.30	4.42	3.461

25/11/74 (40/28 procedure)

Rats. Adult freshly killed
Wt gms.

A I – I	Adult Rat 1	1.7 gm	32 ml
A II – II	Adult Rat 1	1.7 gm	32 ml
H III – III	Adult Rat 1 (20/11 frozen)	1.2 gm	31 ml

PND I	15 ml	LS I	13 ml
PND II	15 ml	LS II	13 ml
PND III	15 ml	LS III	13 ml

A I	3 ml	B I	6 ml	C I	5 ml
A II	2 ml	B II	6 ml	C II	5 ml
A III	3 ml	B III	6 ml	C III	5 ml

Na + K AT Pass (Babram - Kamat - Kaish).
0.1 ml 6-20-8-50 (.5 + .2 + 10 ml water)

1 Na	H I	22.5/0.65	19 Na	LS I	36/0.445	37 Na	44.5/0.35
2 K		19.5/0.71	20 K		23/0.64	38 K	B I 37.5/0.425
3 Na		22.75/0.645	21 Na	LS II	25/0.60	39 Na	48/0.32
4 K		21.5/0.67	22 K		23/0.635	40 K	36.5/0.44
5 Na	H II	22/0.655	23 Na	LS III	38/0.412	41 Na	29/0.54
6 K		22.25/0.65	24 K		28/0.53	42 K	B II 95/0.025 *
7 Na		21.5/0.67	25 Na	A I	41/0.39	43 Na	~ Blanked *
8 K		21/0.675	26 K		36/0.445	44 K	~ *
9 Na	H III	41/0.39	27 Na		41.5/0.36	45 Na	56.5/0.2475
10 K		24/0.62	28 K		36.5/0.44	46 K	B III 40/0.40
11 Na		24/0.62	29 Na	A II	58/0.235	47 Na	56.5/0.25
12 K		26.5/0.575	30 K		52.5/0.28	48 K	38.25/0.4175
13 Na	PND I	41/0.385	31 Na		52.5/0.28 *	49 Na	75/0.125
14 K		40/0.3975	32 K		→ 28/0.55 *	50 K	C I 68.5/0.165
15 Na	PND II	43/0.37	33 Na	(B II)	→ 24/0.625 *	51 Na	73.5/0.1825
16 K		34/0.47	34 K	A III	→ 21.25/0.67 *	52 K	74/0.13
17 Na	PND III	34.75/0.46	35 Na		37/0.43	53 Na	76.5/0.115
18 K		30/0.52	36 K		30/0.52	54 K	C II 68/0.17
						55 Na	78.5/0.105
						56 K	70/0.155

	diff			
A I	0.055 0.06	6.766	3.08	2.196
A II	0.045	5.294	1.6	3.309
A III	0.09	10.59	5.3	1.998
B I	0.075 0.12	11.48	3.46	3.314
B II	—	—	6.63	Out
B III	0.1525 0.1675	18.82	2.98	6.317
C I	0.04	4.707	1.97	2.389
C II	0.055 0.05	6.177	2.12	2.914
C III	0.0425 0.0325	5.589	1.25	4.471

DS57 28 III 24

B II	.09	11.26	6.63	1.697

57	Na		83/0.0825	61	Blank	100/0.0	
58	K	C III	75/0.125	62	1 μM	67.5/0.17	0.17 = 1 μM
59	Na		85/0.07	63	2 μM	46/0.335	
60	K		79/0.1025	64	3 μM	31/0.51	

26/11/74 — Drolein — 1 + 0 + 0.5

1	H I	.02	74/0.13	10.5 mg/ml	25	B I	.04	81/0.09	3.46
2		.02	72/0.1425		26		.04	81/0.09	
3	H II	.02	72.5/0.14	10 mg/ml	27	B II	.04	68/0.17	6.63
4		.02	76/0.12		28		.04	67/0.175	
5	H III	.02	80/0.0975	6.54 mg/ml	29	B III	.04	82/0.085	2.98
6		.02	78/0.1075		30		.04	85/0.07	
7	PND I	.02	87/0.0625	5 mg/ml	31	C I	.08	79.5/0.10	3.94 / 2 = 1.97
8		.02	85.5/0.0675		32		.08	78.5/0.105	
9	PND II	.02	86/0.065	4.8 mg/ml	33	C II	.08	76.5/0.115	4.23 / 2 = 2.12
10		.02	87/0.06		34		.08	78.5/0.105	
11	PND III	.02	87/0.06	6 mg/ml	35	C III	.08	85/0.07	1.25
12		.02	80/0.095		36		.08	87/0.06	
13	LS I	.04	77/0.115	4.6 mg/ml	37	Blank		100/0.0	.065 = 100 μg
14		.04	74/0.13		38	50 μg		92/0.035	
15	LS II	.04	73/0.1375	5.3 mg/ml	39	100 μg		87/0.06	
16		.04	72/0.145		40	150 μg		79/0.1025	
17	LS III	.04	76/0.12	4.42 mg/ml	41	200 μg		76/0.125	
18		.04	77.5/0.11		42	250 μg		69/0.1625	
19	A I	.04	83/0.08	3.08 mg/ml					
20		.04	83/0.08						
21	A II	.04	90/0.045	1.6					
22		.04	91/0.04						
23	A III	.04	71.5/0.145	5.3					
24		.04	73/0.1375						

Repeat Na-K ATPase ~~at pH 7.4~~ ~~Tris~~ (2 ~~x~~) (TD = PH 7.4)

1	H II	Na	68.5/0.165	pH 7.4 by pH labe Broad lines and make to pH 8.6
2		K	84/0.075	
3	H III	Na	88/0.055	
4		K	87/0.06	
5	B II	Na	90/0.045	
6		K	90/0.045	
7	B II	Na	91/0.04	
8		K	87/0.06	

27/11/74

Monkey's freshly killed.

Young monkey (male) 2 kg + Adult 5 kg.

Young monkey (2 kg) Cerebellum (part – ½), Motor cortex (cleaned from) Cotton for expts

---

Young monkey (male) 2 kg m.

Homogenization

I Motor Cortex (Fresh) – 1.6 gm 40 ml H I

II Cerebellum (Fresh) – 1.6 gm 43 ml H II

III ~~Motor~~ Cortex Cerebral } Frozen for 2 months – 2.9 gm 44 ml H III

PND I – 35 ml } rough. (Try not to create achiral vacuum)
PND II – 35 ml }
PND III – 60 ml

[40/28] Total time 60 mts

LS I 12 ml A I ~ B I 4.5 C I ~

LS II 12 ml A II ~ B II 4.5 C II ~

LS III 12 ml A III ~ B III 4.5 C III ~

Asp 133.1

.1 M : 13.31

.05 M : 6.655

1 ml : 6.655 mg

2 ml : 13.3 mg.

Pro . 115.1

.05 M 2 ml = 11.5 mg.

28/III/74

I Rats B I B II B III Combined dialysed 20 hrs
Q 5/II 5 - Tris pH 7.4

II Monkey 27/III B I Dialysed

Asp
Glu
Pro } 0.05 M .5 ml to 2.5 ml. final 10 mM

Ala
GABA
Gly } 0.25 M .5 ml to 2.5 ml final 50 mM.

Protein Rx 2.5 mg/ml. (comm.).
Dilute 5 fold in Pyrex.
(2 ml → to 8 ml) stock (2 — 2.5 ml)

ANS 100 µg/ml. .5 ml to 8 ml 20 fold

Rats } 5 ~~2.5~~ ml. Mouse
Primates } 1.25 ml Tris or TNS
~~1.6 25~~ ml Asp/p. } stock soln.
13.75

2 ml + 0.5 ml Salt
& Protein

5 Mouse
1.25 Tris Mg
13.75 Asp

(Mins 5 ml (2.232 mg/ml)
+ 1.25 ml TNS (0.2 mg/ml)
+ 13.75 Buffer.) = I

RATS - TNS

II = 2 ml + 0.5 ml salt soln.
I

λ	BLANK	Glu .05M	Asp .05M	Pro .05M	Blank	Ach .25M	GLY .25M	GABA .25M

Excitation: 327 mμ.

Emm Spectra

Range: 4 × 1/10 | 4 × 1 | 4 × 1

λ	Blank 4×1/10	Blank 4×1	Glu 4×1/10	Asp 4×1/10	Pro 4×1/10	4×1	Blank 4×1/10	Blank 4×1	Ach 4×1/10	Ach 4×1	GLY 4×1/10	GLY 4×1	GABA 4×1/10	GABA 4×1	λ
350	3.5,	35	3	3	3.5,	35	34		37		35		36		350
360	1 ,	11	1	1	1 ,	11	11		12.5		11.5		12		360
370	1	10	1.5	2	1 ,	10	10		11		10.5		11		370
380	1.25,	13	5.5	5.5	1.25	13	13.5		15.5		14.5		15.5		
390	2.25,	23	19	18.5	2 ,	22.5	22		25		23		25		
400	3.5,	36	39	38	3.5,	35.5	34.5		40		37		40.5		
10	5,	50	74	62	5,	49	48		56		51		56.5		
20	6 ,	60	80	78	6,	59	59		70		62.5		70		
30	6.5,	63.5	87.5	87	6.5,	63	63.5		75.5		66.5		74		
40	6.5	60	86	84.5	6,	60	62		75		65		72		
50	5.5	54.5	76.5	76	5.5,	54	55.5		67		58		64.5		
60	4.75,	46	64.5	64	4.5,	46	47		58		49.5		54.5		
70	4,	39	53	52.5	4,	38	39.5		47		41.5		46		
80 →	3,	30	40.5	40	3,	30	31		37.5		32		35.5		
90 →	2.25,	22.5	29.5	29	2.25,	22	23		27.5		24		26.5		
500 →	2,	17.5	22	21.5	1.75,	17	17		20.5		18		19.5		500
10 →	1.25,	12	15	15	1.25,	12	12.5		15		13		14		510
20 →	1 ,	9	10.5	10.5	1,	9	9		11		9.5		10.5		520

Repeated Ach, GLY, GABA same type. 390-470

Blank			
63.5	67	73.5	

$\frac{.2 \times .25 \times}{316 \quad 20 \times 16}$ $\qquad$ $\frac{.2}{4996} \times \frac{4}{5}$

$\frac{.8}{4996 \times 5}$ $\qquad$ $\frac{.8}{25000}$

$\frac{.8}{25}$ mM.

$\frac{8.}{250}$ mM. $\qquad$ 32 μM.

Ans: $\frac{4.46}{2.232 \times \frac{5}{20} \times \frac{4}{5}}$

$\frac{}{5}$

Ans: 450 μS/mL

ANS: 32 μM.

ANS - Rats

Mix 5 ml (2.232 mg/ml) + 1.25 ml ANS (0.2 mg/ml) 13.75 ml borax
2 ml → 2.5 ml.

Excitation: 390 mμ.
Range: 4 × 1/10, 4 × 1 range.

λ	Blank	Ala	Asp	Pro	Blank	Ach	Gly	Gamma	λ
λ	Blank 4×1/10 4×1	Ala 4×1/10	Asp 4×1/10	Pro 4×1/10 4×1	Blank 4×1	Ach 4×1	Gly 4×1	Gamma 4×1	λ
420	2, 18.5	7	7	2, 18.5	18.5	23	21	21.5	420
430	1.75, 17	16	15	1.75, 17	15.5	20	18	19.0	
440	2, 19.5	26.5	25	2, 19	19	23.5	21	22	
50	2.5, 23	38	36	2.5, 23	23	28.5	25	27	
60	3, 26	46	43.5	3, 25.5	25.5	31.5	27.5	29.5	
70	3, 27	49	46.5	3, 26.5	26	33	28.5	31	
80	2.5 25.5	47	45	2.5, 25	25	31	26.5	29	
90 ↓									
500 ↓	2.5, 22.5	41.5	39.5	2.25, 22	22.5	28	24	25.5	
10	2, 19	35	33	2, 19	19	24	20.5	22	
20	1.5, 15.5	27	25	1.5, 15	16	19.5	16.5	18	
30	1.25, 12.5	20	19.5	1.25, 11.5	12.5	15.5	13.5	14.5	
40 ↓	1, 9.5	15	14	1, 10	9.5	12	10	11	
	.75, 7	10	10	.75, 7	7.5	9	8	8	540
					6	7	6	6.5	550

Repeated. 390-470 (Gamma Gly only)

Blank	Gly	Gamma
26	29	32

I (5 ml amino (2.41 mg/ml)
+ 1.25 ml TNS (0.2 mg/ml)
Monday - TNS + 13.75 ml buffer)
(2 ml I + 0.5 ml salt)

Excitation 327

Range · $2 \times \frac{1}{10}$, 2×1

Recorder
$2 \times \frac{1}{10} = \frac{1}{10}$ (2×1)

λ Blank Glu Asp Pro Blank Ach Gly GABA λ

λ	Blank Glu	Asp Pro	Blank	Ach	Gly (Kom)	GABA	λ
	$2 \times \frac{1}{10}$ 2×1	$2 \times \frac{1}{10}$	$2 \times \frac{1}{10}$	$\frac{2 \times 1}{10}$ 2×1	2×1	2×1	2×1	2×1	
350	3.25, 32	3	3	3.25, 31	25	31.5	27	27.5	350
60	1, 10.5	1	1	1, 10	10.5	13	11	11.5	
70	1, 9	1.5	1.5	1, 9	9	11.5	<10	>10	
80	1.25, 12	4.5	5.5	1, 11.5	11.5	14	12	12.5	
90	2, 19	12	15	2, 18.5	18	22.5	14.5	14.5	
400	3, 29	24	31	3, 28.5	28	34.5	29.5	30	
10	4, 40	38.5	48	4, 38.5	38.5	47.5	40.5	40.5	
20	5.25, 50	50.5	63	5, 47	47.5	58	49.5	49.5	
30	5.5, 53	55	69.5	5.5, 50	50.5	63	53	53	
40	5.25, 51	54	68	5, 49	50	62	52	52	
50	4.5, 45.5	48	60	45, 43.5	44	55	46	46	
60	4, 40	40	50	4, 37.5	38.5	48	40	40	
70	3.5, 33	33	41	3.5, 31	32.5	41	34	34	
80	2.75, 25	25	31	2.5, 24.5	25	31.5	26	26.5	
90	2, 19	18	23	2, 18.5	19	24	19.5	20	
500	1.5, 15	13.5	16.5	1.5, 14	14.5	18	15	15	
10	1 10.5	9.5	11.5	1, 10	11	13.5	11.25	11.25	
20	.75, 7.5	6.5	8	.75, 7.5	8	10	8	8	520

M

Ans $\frac{.2}{316} \times \frac{1.25}{20}$

Ans 32 μM

$2.41 \times \frac{5}{20} \times \frac{4}{5}$

5

482 μg/ml

Monley ANS

Excitation 390

Range: $3\times\frac{1}{10}$, 3×1

Reading $3\times1 - \frac{1}{10}(3\times1)$

T	Blank $3\times\frac{1}{10}$	Blank 3×1	Glu $3\times\frac{1}{10}$	Asp $3\times\frac{1}{10}$	Pro $3\times\frac{1}{10}$	3×1	Blank 3×1	Ala 3×1	Gly 3×1	Ala 3×1	T
420	3	30	11	10.5	3	24.5	29	37	32	33	420
430	3	26.5	23	21.5	3	26	26	33	28.5	29.5	430
40	3.25	30	38	36	3.25	29.5	29.5	36.5	32	32.5	440
50	4	36	55	52	3.75	35	35.5	43	38	38.5	450
60	4.25	40	66	63	4.25	39	39.5	47.5	42	42	460
70	4.5	41.5	72	68.5	4.25	40.5	41.5	49.5	43.5	44	470
80	4.25	40	69.5	66	4.25	38.5	40	47.5	41.5	41.5	480
90	4	35.5	62	59	3.75	34.5	36	42.5	37	37	490
500	3.25	30	51	48.5	3	29	31	36.5	31.5	31.5	500
10	3	26	40.5	38.5	2.5	24	25	30	26	26	510
20	2.5	22	30.5	29	2	19	20.5	24	21	21	520
30	1.5	15.5	22	21	1.5	15	15.5	16.5	16	16	530
540	1	12	16	15	1.25	12	12	14.5	12.5	12.5	540

$N_2^+ - H_2^+$ ATPase

	Diff	μMoles/mc/Hr	Correcting / mc	Activity μMoles/Hr/mg protein
H I	0.0525 | 0.035	5.476	8.57	0.639
H II	0.075 | 0.09	10.31	8.035	1.283
H III	0.015* | .055	6.877	11.25	0.6113
PND I	0.06	7.501	4.643	1.615
PND II	0.08	10.0	6.07	1.647
PND III	0.165	20.63	6.607	3.123
LS I	0.12	15.01	12.5	1.2
LS II	0.155	19.37	8.57	2.261
LS III	0.2125	26.57	10.357	2.564
A I	0.0075	0.9378	1.339	0.6703
A II	-ve .0025	out	1.875	out
A III	0.035	4.376	0.625	6.701 ?
B I	0.11 | 0.12	14.37	6.428	2.238
B II	0.1175 | 0.095 | 0.105	13.23	4.553	2.905
B III	0.1075 | 0.1125	13.75	1.964	6.701 ?
C I	0.02	2.5	1.428	1.751
C II	0.0475	5.939	1.785	3.328
C III	-ve 0.005	out	0.58	out
B II (R) (25 III 14)	0.09	11.26	6.63	1.697

Coleman 5-40 mpx Monkey's

28/11/74 $Na^+ + K^+$ ATPase 27/11/74 Monkey's pregn.

0.05 ml enzyme 8–8-30 pm + .5 + .2 + 5 ml H_2O

30 only membranes

HI M	1	Na	45	/0.3475
	2	K	40	/0.40
	3	Na	41	/0.385
	4	K	38	/0.42
HII M	5	Na	50.5	/0.295
	6	K	42.5	/0.37
	7	Na	48	/0.32
	8	K	39	/0.41
HIII M	9	Na	27	/0.57
	10	K	26	/0.585
	11	Na	28	/0.555
	12	K	24.5	/0.61
PNDI M	13	Na	68.5	/0.165
	14	K	59.5	/0.225
PNDII M	15	Na	69	/0.16
	16	K	57	/0.24
PNDIII M	17	Na	68.5	/0.165
	18	K	46	/0.33
LSI M	19	Na	40.5	/0.39
	20	K	31	/0.51
LSII M	21	Na	53.5	/0.27
	22	K	37.5	/0.425
LSIII M	23	Na	58	/0.2375
	24	K	35	/0.455
AI M	25	Na	64.5	/0.19
	26	K	63.5	/0.1975
AII M	27	Na	62.5	/0.205
	28	K	63	/0.2025
AIII M	29	Na	88.5	/0.055
	30	K	81	/0.09
BI	31	Na	39	/0.41
	32	K	30	/0.52
BI	33	Na	40	/0.40
	34	K	30	/0.52
BII	35	Na	44	/0.3575
	36	K	33.5	/0.475
BII	37	Na	44.25	/0.3575
	38	K	35.5	/0.45
BII	39	Na	46	/0.335
	40	K	36	/0.44
BIII	41		74.5	/0.1275
	42		58	/0.235
BIII	43		75.5	/0.1225
	44		58	/0.235
CI	45		83.25	/0.08
	46		79	/0.10
CII	47		81.75	/0.0875
	48		73	/0.135
CIII	49		91.5	/0.04
	50		92	/0.035
BII(R) 25/III/74	51		35.5	/0.45
	52		29	/0.54
Blank	53		100	/0.0
1 μM	54		47.5	/0.325
2 μM	55		24	/0.62
3 μM	56		11	/0.96
4 μM	57		6.75	/1.175

*

28/29 (11/74) Protein — Coleman 5Z10 comp — 27/11 preparation (1ml + .5 + 5)

H I	.021	75	0.125
	.022	77	0.115
H II	.023	76	0.12
	.024	78	0.105
A III	.025	69	0.16
	.026	70	0.155
PND I	.027	86	0.065
PND II	.028	82	0.085
PND III	.029	81	0.0925
LS I	.0210	67	0.175
LS II	.0211	75.5	0.12
LS III	.0212	71.5	0.145
A I	.0813	84	0.075
A II	.0814	76.5	0.105
A III	.0815	92	0.035
B I	.0416	64	0.195
	.0417	68.5	0.165
B II	.0418	76	0.12
	.0419	73	0.135
B III	.0420	88	0.055
	.0421	86	0.055
C I	.0822	63	0.08
C II	.0823	79.5	0.10
C III	.0824	93	0.0325
25 RATS BIGH DIAL	.0425	87.5	0.0575
	.0426	86.75	0.0675
27 III Marilen B I DIALYSED	.0427	85.5	0.0675
	.0428	85.5	0.0675
Blank		29	100 / 0.0
100 µg		30	82.5 / 0.0825 × 1.07 = 100 µg
200 µg		31	72.5 / 0.14

H I	8.57 mg/ml
H II	8.035 mg/ml
A III	11.25 mg/ml
PND I	~~8.8877~~ 4.643
PND II	6.07 mg/ml
PND III	6.607
LS I	12.5 mg/ml
LS II	8.57 mg/ml
LS III	10.357
A I	1.339
A II	1.875
A III	10.625
B I	6.428 ~~6.429~~ 5.89 (lower value) 6.426 (average)
B II	4.553
B III	1.964
C I	1.428
C II	1.785
C III	0.58

Rats DIALYSED

2.232 mg/ml

Morden Dialysed

2.41 mg/ml

13/1/75

Fluorescence studies

Dr. Lecithin : Chyme) : Stock 1.5 mg/ml.

ANS 0.2 mg/ml soln.

I set

8.4 ml buff + 1.2 ml ANS > 2.4
Q.6

Add 2 ml mins + 0.4 ml ANS } : 6 ml + 0.4 ml ANS

1-1 0.6 ml buff + 0.6 ml Ach. + 0.6 ml Gluc + 0.6 ml GABA + 0.2 ml Ach + 0.2 ml Gluc etc

II set

8.4 ml buff + 1.2 ml mins

=> 2.4 ml + 0.6 ml buff. etc

Add

2 ml ANS + 0.4 ml mins >> 0.6 ml + 0.4 ml buff + 0.2 ml buff + 0.2 Ach

etc - - - - -

Range LIPID (Synthetic) Binding

6×1 range

Excitation: 340 Emission: 415/470

Members

Specific

Excitation: (4×1 range)

Emission

Free	GABA	
29	31	420
30	32	30
37	39.5	40
45	48	50
49.5	~~49.5~~ 54	60
52	55.5	70
48.5	52.5	80
43.5	47	90
37	40	500
30	32	10
24.5	26	20
19	20	30
15	16	40
12	12.5	50

Free	Ach	Glu	GABA	lipid added
9.5	15	14.5	17.5	0.0
14	22	59	24.5	+0.05
21	29	57 (5×1)	32	+0.05
25	34	82 (5×1)	39	+0.05
32	37	56 (4×1)	46	+0.05
37	41	67 (4×1)	53	+0.05
43	48	80 (4×1)	60	+0.05
52	57	100 (4×1) 55 (5×1)	72	+0.1
64	65	67 (3×1)	85	+0.1
73	77	76 (3×1)	100	~~+0.1~~
83	89.5	85 (3×1)	65 (5×1)	+0.1
				~~+0.1~~

(6×1 range)

				Ans Ach
7.5	12	10	19.5	0.0
15	21.5	62.5 (5×1) (35) (5×1)	27.5	+0.05
20.5	31.5	60 (5×1)	35	+0.05
28.5	35.5	80 (5×1) 45 (4×1)	38	+0.05
29.5	40	57 (4×1)	47	+0.05
34	47	65 (4×1)	54	+0.05
36	52	78 (4×1)	58	+0.1
45	56	95 (4×1)	66	+0.1
50	64	55 (3×1)	75	+0.1
56	66	64.5 (3×1)	82	+0.1
61	73	72 (3×1)	91	~~+0.1~~
				+0.1

Repeat Incubs

4x1 rxn (With 250 nM 417 GABA in Tris buffer)

Excel 340

λ	Free	GABA
420	20	21
30	22.5	23
40	30	30
50	38.5	38
60	42	42.5
70	45	44.5
80	41	41
90	37	36
500	31	31
10	26.5	26
20	19.5	19.5
30	15	15.5
40	11.5	11.5
50	9	9

14-2-75

4 Rats — Control ~ 6 gm
4 Rats — Low protein ~ 6 gm.
4/10/74 Monkey brain (Cerebellar) ~ 5 gm.

↓ Homogenised — 1000 rpm 10 strokes (motor)
in 10 volumes 8.5% Sucrose in Tris HCl (pH 7.4 10 mM)

Spun 3500 rpm 20 mts
Supernatant spun at 10,000 rpm 30'
Resuspended each set in 15 ml Tris-buffer
Layered over 10 ml 40% Sucrose
spun at 13000 rpm 30'
Sucrose collected & suspended in 10 ml buffer. Dialysed for 48 hrs (many changes)

35/25 gradient 640250 g 60 mts

B I — Control Rats
B II — Expered Rats
B III — Monkey old

↓↓ B I DIALYSED

	Prot.	OD.
B I D	.04 ml	78.5/0.105
B I D	.08 ml	60/0.22
520 µg/ml BSA .1		62/0.085 (.055)
" .2		80/0.095 (.11)
" .3		67/0.175 (.165)
" .4		63/0.20 (.121)
Blank		0/100%

100 µl

Prots 2.5 mg/ml

A-cholinesterase

	0	30	60	90	120	150	180
B I D ①							
②							

18/2/75 BID. Membrane.

ANS Soln : 0.2 mg/ml.

After last addition take specta

I st set

8.4 ml buffer + 1.2 ml ANS
9.6

=> 2.4 + 0.6 buffer (one)
each + 0.6 salts
2.5 ml in cuvette + .05

Addition

2 ml membrane } .6 ml I + .4 ml buffer
+ 0.4 ml ANS II + 0.2 ml buffer + 0.2 ml salt

II nd set-

8.4 ml buffer + 1.2 ml membrane 2.4 + 0.6 buffer
=> (or + 0.6 salts)
like

Addition

2 ml ANS + 0.4 ml => 0.6 + 0.4 buffer
membs
+ 0.2 buffer + 0.2 salt

Rat - ANS

1 A-Choline ①'
2 Butyryl choline chloride
3 Na ①'
4
5 K
6 Li
7 Cs ①'''
8 Ca^{++} ①''''
9 Mg^{++} ①'''''
10 GABA ①'
11 GLY
12 PRO
13 L Glu ①' 10 mM
14 D Glu ①'' 10 mM
15 Glutamine ①' 10 mM
16 L Asp ①''' 10 mM
17 D Asp ①''' 10 mM
18 Asparagine
19 L-Arginine
20 Chlorpromazine
21 Tryptophenazine
22 Novocaine
(23 Noradrenalin)

Start 10 mM

24
6

Two days

English Calcutta

424

Stock Solutions

2 ml.

L-Glu 50 mM.

D Glu 50 mM 14.7 mg in 2 ml 0.05 M

D-Glutamine 50 mM 14.

N Acetyl L-Glutamine 50 mM.

L Asp 50 mM

D Asp 50 mM

D-Asparagine 50 mM

Ach 250 mM

NA 250 mM

GABA 250 mM

Glu 250 mM

Cat+ 50 mM

Mg^{++} 50 mM

L. Asparagine

L. Glutamine. 36.5 mg in 5 ml 0.05 M 35/25 %

Rat 2.5 mg/ml

390 - 470 mems

Cs+

4 X 1 Range 1 Free 2 Ach 3 Na 4 GABA

Free 1	Ach 2	Na^+ 3	GABA 4	Memb added Q Liq add
1.5	2.0	1.5	6.0	0.0
4	5.5	5.5	11	+0.05
7.5	9.5	10	14.5	+0.05
10.5	12.5	14.5	17.5	+0.05
15.5	16.5	18.5	18	+0.05
18.5	19.5	22.5	20.5	+0.05
21.5	22.5	27	23.5	+0.05
27	28.5	35	29	+0.1
32.5	33.5	42	34	+0.1
37.5	39.5	48	39.5	+0.1
41.5 ~~42~~	44.5	54	44	+0.1

~~+0.1~~

(protein added 6 ml) (1)

426 4x1 range 390-470

5 Free	6 Ach	7 Na	8 GABA	ANS added
5.5	6	5	13.5	0.0
10	12	13.5	17.5	+ 0.05
13.5	17	20.5	21.5	+ 0.05
16.5	22	27	24.5	+ 0.05
19.5	26	32	27.5	+ 0.05
22	29	36	30.	+ 0.05
24.5	32.5	41	32.5	+ 0.05
28.5	38	46.5	36	+ 0.1
32	41.5	51	39.5	+ 0.1
35	44.5	54	43	+ 0.1
37.5	46.5	57	4,5	+ 0.1

(1A)

9 – 1 · Free
10 – 2 · L Glutamate
11 – 3 D-Glutamate
12 – L Glutamine.

Mixes added

13
14
15
16
ANS added

U.V

390/470

1 Free (9)	2 L. Glutamate (10)	3 D Glutamate (11)	4 L Glutamine (4×1) (12)

4×1 range

Free	L. Glu	D Glu	L Gln	Gly Membadd
1.5	1.5	1.5	1.5	0.0
4	79.5 (43 (3×1))	64	5	+0.05
6.5	85 (163 (3×1)) 45 (2×1)	68 129	9	+0.05 +0.05
9.5	65 (293 (2×1))	52 191 (2×1)	13	+0.05
13	85 (383 (2×17)) 45 (1×1)	69 259 (2×1)	17.5	+0.05 +0.05
16	99 (374 ~~715~~ 376 (2×114)) 53 (1×1)	82 311 (2×1)	21	+0.1
18	60 (~~454~~ 454 (1×1))	50 374 (1×1)	24.5	+0.1 +0.1
23.5	73 (~~505~~ 556 (1×1))	61 460 (1×1)	31.5	+0.1
28	80.5 (~~430~~ 616 (1×1))	67.5 512 (1×1)	35.5	+0.1
32	89 (~~1111~~ 684 (1×1)) 57 (4×1/10)	74.5 564 (1×1)	40.5	
38	63 (756 (4×1/10))	54 648 (4×1/10)	46	

(2)

505 4
1/811
1210
570

4×1 range 390 - 470 (12) (12)

4×1 = 12 (4×1/10)

(4×1)	(4×1/10)	(4×1/10)	4×1	
Free (13)	L-Glu (14)	D Glu (15)	L. Gln (16)	ANS
4.5 (4×1)	6 (4×1) / 1 (4×1/10)	6.5 (4×1) / 1 (4×1/10)	4.5 (4×1)	added
9 (4×1)	11 (4×1/10) 132	11 (4×1/10) 132	9 (4×1)	0.0
12.5 (4×1)	18 (4×1/10) 216	18.5 (4×1/10) 222	13 (4×1)	+ 0.05
15.5	23.5 (282)	25 300	16	+ 0.05
18.5	28 336	29 348	19.5	+ 0.05
21.5	31.5 378	33 396	22.5	+ 0.05
23.5	34 408	36 432	25	+ 0.05
27.5 (4×1)	39 (4×1/10) 468	41 (4×1/10) 492	30	+ 0.05
30	42.5 510	44.5 534	32	+ 0.1
34	48 576	48 576	37	+ 0.1
37	54 648	51 612	39	+ 0.1
38.5	55.5 666	53.5 642	42.5	+ 0.1

(2A)

last- auch spectra taken

Excitation maximum 390 nm

Emission Spectra 390 nm excitation

	Free (4×1)	L-Glu (4×1/10)	D-Glu (4×1/10)	L-Gln (Glutamine) (4×1)
410	over 100	15.5	13	over 100
20	16	7	6	17
30	19	15	13.5	19
40	24	26	24	25.5
50	31.5	39	36	33
60	36	48	46	38
70	39	53	57.5	42
80	39	53.5	53.5	41
90	35	48	47	36
500				
10	30	40.5	40.5	31.5
20	25	32	33	25.5
30	20	25	25.5	21
40	15	18	18.5	15.5
50	11.5	13	13.5	12
	8.5	9	9.5	9

0.2 mg/ml × 100 µg/mg ⇓ ×318.5 µ Moles ANS range

	A 401	A 401	Conc	Reciprocal
1/51	2.2924	0.7948	6.234	0.1604 (0.1604)
2/52	2.5850	1.0874	12.23	0.08177
3/53	2.7528	1.2552	18.00	0.05556
4/54	2.8694	1.3720	23.55	0.04246
5/55	2.9586	1.4610	28.91	0.03459
6/56	1.0300	1.5324	34.07	0.02931
8/58	1.1392	1.6416	43.81	0.02282
10/60	1.2218	1.7242	53.00	0.01887
12/62	1.2888	1.7892	61.55	0.01625
14/64	1.3399	1.8423	69.55	0.01438

Protein

original 2.5 mg/ml protein

1250 µg/ml. (1.25 mg/ml)

	Conc µg/ml	$\times 10^{-3}$ Reciprocal (mg/ml)$^{-1}$
1/5	24.51	40.8
1/10	48.07	20.8
1/20	70.78	14.12
1/30	92.53	10.81
	113.6	8.804
	134.0	7.463
	172.2	5.807
	208.3	4.801
	241.8	4.136
	273.4	3.659

Mem added

17 Free — Ans 21

19 L Asp 1 mM — 22

18 D - Asp. 1 mM. — 23

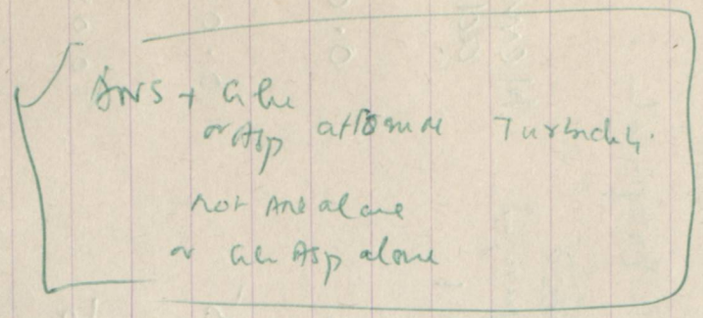
20 L Asparagine 1 mM — 24

Mem added	Mem added
~~K+ 25~~	~~433~~ 34
Acl 26	30
Ca++ 27	31 hrs.
Mg++ 28	32 Reagt
K+	

19/2/75 390 / 470 Ans 18 12 Prepared.

(17) Free	(18) L Asp	(19) D Asp	(20) L Asparagine	Mem added
4×1	4×1/10	4×1/10	4×1	
1.5	2.5 (4×1) + 0.5 (4×1/10)	2.0 (4×1) + 0.5 (4×1/10)	2	0.0
5	8.0 (4×1/10) + 8.1 (4×1)	6.5 (4×1/10) + 8.2 (4×1)	5.5	+0.05
8	15.5 (186)	16 (192)	8.5	+0.05
11	21.5 (258)	22.5 (270)	12	+0.05
14	27 (324)	28 (336)	15	+0.05
16.5	32.5 (390)	33 (396)	17.5	+0.05
				+0.05
19.5	37 (444)	38 (456)	21	+0.1
25	43.5 (522)	44 (528)	26.5	+0.1
30	49.5 (594)	49.5 (594)	31.5	+0.1
34.5	55.5 (666)	55 (660)	37	+0.1
38.5	59 (708)	58.5 (702)	41	

(3)


Tyr Ser + Glu
or Asp at Ser 214 Turkeys.
not Ans alone
or Glu Asp alone

Ready in 4×1 = 4

Reagin 10(4+1/10) = 435

Rat brain 2-5 mg/ml homog

390/470

Free 4×1	L·Asp (4×1/10)	D-Asp (4×1/10)	L Asparagine (4×1)	ANS added
5 (4×1)	5·5 (4×1) 1< (4×1/10)	4·5 (4×1) 1< (4×1/10)	4·5	0·0
9·5 (4×1)	12·5 150 (4×1/10)	12·5 150 (4×1/10)	9 (4×1)	+ 0·05
12·5	19·5 234	20 240	13	+ 0·05
15·5	25 300	28 336	16·5	+ 0·05
18·5	30·5 366	33 396	19·5	+ 0·05
21	35 420	37 432	22·5	+ 0·05
23·5	39 468	40 480	25	+ 0·05
27·5	45 540	45 540	29	+ 0·1
31	49 588	48 576	32·5	+ 0·1
34	53 636	51 612	35·5	0·1
37·5	57 684	53·5 642	40	0·1

3A

Membadd. ANSadd.

Hcl	25	29	(Twice what in Cl$_2$, OSP - -)
Ca^{++}	26	30	50mM ⇒ half 10mM
Mg^{++}	27	31	50mM ⇒ 10mM
K^+	28	32	250mM ⇒ 50mM

4x1 range.

Hcl (25)	Ca^{++} (26)	Mg^{++} (27)	K^+ (28)	Memb added
2	2.5	2	2	0.0
84 (4x1)				
9	12	10	7	0.05
17	22.5	21	12	0.05
23	34.5	26.5	17	0.05
29	44	35	22	0.05
35	53	42	27	0.05
40	61.5	48.5	31.5	0.05
46.5	75.5	61.5	40	0.1
52	88 (4x1) 48 (3x1)	71	47	0.1
—	96	80	53	0.1
—	100 (4x1) 58.5 (3x1)	89	59	0.1
—	111			

(4)

4x1 mg

390-470 ANS

4x10 HCl (29)	Ca^{++} (30)	Mg^{++} (31)	K^{+} (32)	ANS added
6	6.5	6	4.5	0.0
11	21	18.5	12	0.05
19	34	29.5	18.5	0.05
24.5	43.5	38	23	0.05
29	52	46.5	28	0.05
33	61	54	33	0.05
36	71.5	60	37	0.05
41	78.5	69	41	0.1
45	84.5	77	46	0.1
47	89	82	48.5	0.1
50	96	87	52	0.1

4 cl more than twice as much as needed to dissolve my sample as added to the cuvette.

(4A)

protein
2.5 mg/ml
441

4 x 1 rxc.

20/2/75

(33) Free (34) Cs (35) Li (36) Ach. Rat (1812) brain

4X1 range 390 / 470 Brs

Free	Cs	Li	Ach.	Membadded
1.5	1.5	1.5	1.5	0.0
4.5	6	6.5	6	+0.05
7.5	10.5	12	10	+0.05
11	15	16.5	13.5	+0.05
13.5	19.5	22.5	17.5	+0.05
19.5	24.5	27	22.5	+0.05
21	28.5	32	26	+0.05
25	37	41	32.5	+0.1
30	46	51	40	+0.1
35	52	58	47	+0.1
39.5	59	66	53	+0.1

(5)

19 Cl. 3
746.3

4X1 mc 390/470 | Rel- 2.5 mg/ml
ANS .2 mg/ml

37	38	39	40	ANS added
Free	Cs	Li	Adh	
5	5	5	5	0.0
9.5	12	12.5	11.5	+0.05
13.5	17.5	19	16.5	+0.05
17	23	25	21.5	+0.05
20.5	28	30.5	26	+0.05
23	32	34.5	29	+0.05
26	36	39	33	+0.05
30.5	43	46.5	38.5	+0.1
35	48.5	52.5	44	+0.1
38	54.5	58.5	47.5	+0.1
41.5	57.5	62	51.5	+0.1

(5A)

All Abbreviations as usual

See 1. Lymphocyte plasma membranes. I
Thymic & splenic membranes in
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modification of receptor
944-46. E.M. Landau D. Bentaiam Science 185(4153)

3. Antipsychotic drugs and dopamine stimulated
adenylate cyclase prepared from corpus striatum
of rat brain (neuroleptic drugs-/drug analogs)
PNAS 71(7)

4. Inhibition of acetylcholine binding to
a dimyristoyl-x lecithin bilayer.
(Lipid bilayer / Ion Trap)
Tian Yow Tsong PNAS 71(7)

5. Cholesterol lipid bilayer interactions
Normal effect on biological membranes
Jump A.G. Lee* N.M. Birdsall J.C. Metcalfe P.N.T. Toon A. Warnen
Fig A.G. Lee Biochemistry 13 (18)

6. The production of pantheonopic mammalian
RR embryos and their use in biological research
Biological Reviews 49(3)
C.F. Graham Inst. Oxford. Dept Zool
PARKS Road
Oxford. England

Φ

1½

20 µg/ml TNS

200 µS/ml TNS

protein

Ile ARGININE Asn

16

DIC RNS

TNS m'lu

[illegible]

[illegible]

Kalidasa'

I would be his laughing stock

~~Been~~ Lacking as it is discretion, what-soever

I prattle ~~like~~

~~The shall killed~~

"I become his laughing stock if his uncalled

duca duce ~~modified~~ ~~[illegible]~~ ~~[illegible]~~ pack-

saddle-horse

a might ~~[illegible]~~ speed to reach a

a fort accessible on horseback

as they might from a rayless

& bright Career

((The Colonys of

we dream are

or [illegible] Real if

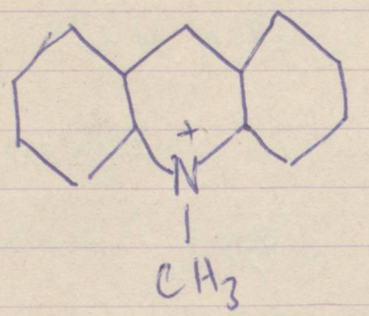
not before))

Fabulous Explis design

472

Bacteriostasis

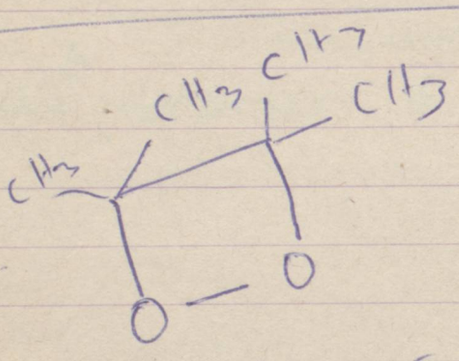
(1)



Acridomium Smelly
Fluorescence in H_2O
Fluorescence Enriched
in non polar solvents

Affinity to D. choleraeae & Lés

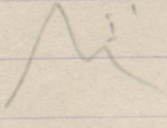
(1)



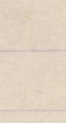
Dioxetène

Thermal & photochemical
decomposition to two aldehyde
excited products

Nachmanson
Nature Biochem
1972.
Ach. via Ca^{++}
activ.
by Ca dependent
calmodulin

(3) Pyrene → 

pyrene
fluorescence
in water



as
molecular
mobility

Incl-Calcium of in label

Expt. Naja Naja

Purified venom

Lecithin

cephalin

Vortex mixer.

Homogenizer.

(1) Fluorescent Cuvettes 4

Polarimeter tubes 4

Spectrum:
- DNP Peptide repeats
- Lecithin 1 repeat

IR Do IR's for the PAS. etc

Fluorescence Thorough at PH's

3, 5, 7, 9.

Stern Conc. in Tris buffer

Further

(1) Compare BSA | Oval albumin Prolein estimation.

(2) Effect of Urea on SDH. Urea in Rats
Urea in Monkey

(3) Solubility studies in Hexane/Ether.

KRLE Van Holde (Phy Biochem)

Mahadevan Watson (Mol Biol of Gene)

I
Ach
Gaba
Serotonin
N.A.
} 0.5 - 1 mg/ml.
+ 2.5 mg albumin solution
for interaction studies

II
TNS
ANS
DNS
ANSA
} in D_2O ~ 0.5 - 1 mg/ml
+ 2-5 ml albumin solution
+ ~ 7/100 mg
(Preparation of Lipids)

III
TNS
DNS
DNS
} → Probably absorbed ORD.
Treat with albumin. Separate
Na Ioma } Lipids (Sonicate)

IV Activity } Na⁺K⁺ ATPase
Ca⁺⁺ ATPase
Adenylate cyclase
} in Presence of Ach, NA
(after Care)
effect

V Lipids → Phase transition
Temp. Effect Change in Ach
Ach + Lipid Specific Chym.
Ach → DNS, TNS binding sites
Ach membrane Temp Phase trans.
Ion Effects

VI Ach & Purification WORD.
Direct & Indirect

VII Ach Lipids Without de...
Probe Specific → Lipid added

VIII Peptides + Lipids near POLY

AA	Sample III %	Sample IV %
ays Acid	1.107	1.687
ASP	7.995	8.896
Thr	3.101	2.861
Ser	2.032	1.469
Glu	11.47	10.91
Pro	4.665	4.812
Gly	5.003	7.034
Ala	10.53	7.603
Val	5.316	5 927
aysm	—	—
Met	.3884	.5640
Ile	5.299	5.728
heu	9.423	9.399
Thr	2.168	2.319
Phe	3.383	3.450
Lys	6.4	5.406
His	1.92	2.074
Amm	15.37	15.33
Arg.	4.394	4.835

A.A.	Sample III Area	Area/Color Conc.	Sample IV Area	Area/Color Conc.
Cys. Acid	18	2.124	22	2.596
Asp	130	15.34	116	13.69
Thr	50	5.95	37	4.402
Ser	30	3.9	20	2.260
Glu	184	22.5	140	16.80
Pro	17	8.95	14	7.406
Gly	78	9.6	88	10.82
Ala	120	20.2	100	11.7
Val	85	10.2	76	09.12
Cysn	NIL	—	NIL	—
Met	6	0.745	7	0.868
Ile	90	10.17	78	8.814
Leu	160	18.08	128	14.03
Tyr	35	4.16	30	3.570
Phe	55	6.491	45	5.310
Lys	118	12.28	80	8.320
His	33	3.761	28	3.193
Ammon	250	29.49	200	23.60
Arg	68	8.431	60	7.440
		191.872		93A

I Ann. Rev. Pharmacology. (1975)

① Changeux : Cholinergic Receptor.

② DeFeudis : Amino acids as central neurotransmitters.

II BSA – SDS.

① Halfman BBA 243, 294 (1971).

② Magnuson JACS 94, 5461 (1972) – ^{35}Cl NMR study.

III Effect of Tris & Histidine on human erythrocytes

Hanahan BBA 382, 634 (1975).

IV ⓐ EPR of str. perturb by 3-methyl indole in the protein & lipid region of erythrocytes.

Bray. BBA 382 534 (1975)

ⓑ Int. bet. proteins & detergents which contain a hydrocarbon chain longer than 16 C-atoms.

A. Ushiwata BBA 393, 215 (1975).

ⓒ Phosphatidyl-choline – BSA interaction.

A. Jonas: BBRC 64 1003 (1975).

(5) Rb scale (0-2)
WL-23
WL-23
WL-23

Blue book pgs 191 - Thin book Pgs 39

TNS Rats: 360/430 TNS: 57 MMols

[Protein mg/ml]$^{-1}$ ⇓ μg		ΔF Free	$\frac{1}{\Delta F}$	ΔF Na	$\frac{1}{\Delta F}$	ΔF Ach	$\frac{1}{\Delta F}$	ΔF GABA	$\frac{1}{\Delta F}$
17.69	56.538	9	.111	11	0.091	12	0.0835	8	0.125
34.69	28.82	18.5	.054	21.5	0.0465	27	0.037	15.5	0.0645
51.09	19.59	28	.0357	33	0.0304	41.5	0.0241	23.5	0.0425
66.80	14.97	37	~~.027~~ .027	42.5	0.0236	56	0.0179	31	0.0322
82.04	12.19	45	.0222	52	0.0192	70	0.0142	37.5	0.0267
96.70	10.34	55	.0182	59.5	0.0168	86.5	0.0116	44	0.0228
111.1	9.001	61.5	.0165	69.5	0.0144	110	0.0091	52	0.0193
~~111~~ 137.6	7.266	75	.0133	~~84~~	0.0116	145	0.0069	61	0.0164
162.8	6.140	87	.0115	96	0.0104	182	0.0055	73	0.0137
186.2	5.370	100	.01	112	0.0089	250	0.004	84.5	0.0119
208.3	4.801	116	.0086	130	0.0077	260	0.00385	100	0.01

TNS / Rat 360-430 TNS added on Membrane 180.5 µg/ml

[TNS]	$[TNS]^{-1}$	ΔF Free	Reciprocal ΔF^{-1}	ΔF Na⁺	ΔF^{-1}	ΔF Ach	ΔF^{-1}	ΔF GABA	ΔF^{-1}
5.588	0.1789	13	0.077	18	0.0555	18	0.0555	12	0.0865
10.96	0.09119	23.5	0.0425	33	0.0302	34.5	0.029	21	0.0475
16.13	0.0620	33	0.0302	45.5	0.022	47	0.0213	31	0.0322
21.1	0.04734	42	0.0238	58.5	0.0171	60	0.0167	37.5	0.0267
26.9	0.03861	49	0.0204	66.5	0.015	68	0.0147	42	0.0238
30.54	0.03275	56	0.0179	74.5	0.0134	75	0.0134	47	0.0212
34.99	0.02862	62	0.0162	83.5	0.012	84.5	0.0118	55	0.0182
43.46	0.02301	69.5	0.0144	93.5	0.0107	92	0.0109	60	0.0167
51.40	0.01946	78	0.0128	103.5	0.00965	97.5	0.0103	65.5	0.0153
58.8	0.01701	83	0.012	109.5	0.00915	106.5	0.0094	70	0.0143
65.77	0.01520	99	0.0101	119.5	0.00835	108.5	0.0092	75	0.0134

Map 17

TNS / Rat- TNS Added

TNS [TNS]$^{-1}$	Free ΔF ΔF^{-1}	Ach ΔF ΔF^{-1}	Hhy ΔF ΔF^{-1}	ChCl ΔF ΔF^{-1}
5.588/0.1789	19 0.0525	13 0.077	11.5 0.087	14 0.0715
10.96/0.09119	32 0.0313	26 0.0386	21 0.0475	25.5 0.0392
16.13/0.0620	41 0.0244	37.5 0.0267	30.5 0.0328	38.5 0.026
21.1/0.04739	51 0.0196	51 0.0196	38 0.0264	50.5 0.0198
25.9/0.03861	59.5 0.0168	64 0.0156	45 0.0223	56.0 0.0179
30.54/0.03275	67 0.0149	73 0.0137	52 0.0192	64.0 0.0156
39.26/0.02548	77 0.013	84 0.0119	60.5 0.0165	75 0.0133
47.49/0.02106	88 0.0114	100 0.01	69 0.0145	87 0.0115
55.16/0.01813	94 0.0106	110 0.0091	76 0.0132	89 0.0112
62.33/0.1604	104 0.0096	124 0.00805	87 0.0115	97 0.0103
69.06/0.01447	106 0.00945	127 0.0079	86 0.0116	98 0.0102

(Take gly readings)

TNS / Rat Protein addect

Protein µg/ml	[Protein]$^{-1}$	ΔF	ΔF^{-1}	ΔF	ΔF^{-1}	ΔF	ΔF^{-1}	ΔF	ΔF^{-1}
		Free		Ach		Gly		Chol	
77.69	56.53	8.5	0.118	14.5	0.069	8.5	0.118	9	0.111
34.69	28.82	16.5	0.0605	28	0.0358	16	0.0625	18.5	0.054
51.07	19.59	24	0.0417	40.5	0.0247	24	0.0416	27	0.0371
66.80	14.97	31.5	0.0318	53.5	0.0187	31	0.0322	35.5	0.0282
82.04	12.19	38.5	0.026	66.5	0.0151	37.5	0.0269	43.0	0.0242
96.70	10.34	45.5	0.022	81.5	0.0123	44.5	0.0223	52	0.0193
111.1	9.001	51.5	0.0194	94.5	0.0106	50.5	0.0198	58	0.0173
137.6	7.266	61	0.0164	123	0.00081	60.5	0.0165	70.5	0.0142
162.8	6.140	72.5	0.0138	142.5	0.007	71	0.0141	82	0.0122
186.2	5.370	82	0.0122	178.5	0.0056	82	0.0122	93.5	0.0107
208.3	4.801	90	0.0111	218.5	0.0046	91	0.0109	103	0.0097

18/2/75

ANS added Curve

Protein ~~45~~ 250 µg/ml ✓ DO (1A)

ANS	$[ANS]^{-1}$	ΔF FREE	ΔF^{-1}	ΔF ~~Acet~~ Ach	ΔF^{-1}	ΔF Na^+	ΔF^{-1}	ΔF GABA	ΔF^{-1}
6·234	0·1604	~~25~~ 4·5	0·222	6	0·167	8·5	0·117	4	0·25
12·23	0·08177	~~6~~ 8	0·125	11	0·091	15·5	0·0645	8	0·125
18·00	0·05556	~~8~~ 11	0·091	16	0·0625	22	0·0455	11	0·091
23·55	0·042 46	~~10~~ 14	0·0715	20	0·050	27	0·0371	14	0·0715
28·91	0·03459	~~12~~ 16·5	0·0605	23	0·0435	31	0·0323	16·5	0·0605
34·07	0·02935	~~26~~ 19	0·05 25	26·5	0·0378	36	0·0276	19	0·0525
43·81	0·2282	~~25·5~~ 23	0·0435	32	0·0313	41·5	0·0241	22·5	0·0445
53·00	0·01887	26·5	0·0378	35·5	0·0282	46	0·0217	26	0·0385
61·55	0·01625	29·5	0·034	38·5	0·026	49	0·0202	29·5	0·034
69·55	0·01438	32	0·0312	40·5	0·0247	52	0·0192	31·5	0·0318

DO

(GABA to ANS binding GABA to membrane bonds to be run + on desk)

(24)

Fre	L-Glu	D-Glu	L-Glutamine
4.5 0.222	131 0.0076	131 0.0076	4.5 0.222
8 0.125	215 0.0047	221 0.0045	7.5 0.133
11 0.091	281 0.0036	299 0.0033	11.5 0.087
14 0.0715	335 0.003	347 0.0029	15 0.0665
17 0.059	377 0.0027	395 0.0025	18 0.0555
19 0.0527	407 0.0025	431 0.0023	20.5 0.0484
23 0.0435	467 0.0021	491 0.002	25.5 0.0392
25.5 0.0393	509 0.002	533 0.0019	27.5 0.0364
29.5 0.0339	575 0.0017	575 0.0017	32.5 0.0308
32.5 0.0308	647 0.0016	611 0.0016	34.5 0.029
34 0.0294	665 0.0015	641 0.0016	38 0.0264

3A

Free	L-Asp	D-Asp	L-Asparagine
4.5 0.222	150 0.0017	150 0.0067	4.5 0.222
7.5 0.133	234 0.0043	240 0.0042	8.5 0.118
10.5 0.095	300 0.0033	336 0.003	12 0.0435
13.5 0.074	366 0.0027	396 0.0025	15 0.0666
16 0.0625	420 0.0024	432 0.0023	18 0.0555
18.5 0.054	468 0.0021	480 0.0021	20.5 0.0487
22.5 0.0445	540 0.0019	540 0.0019	24.5 0.041
26 0.0385	588 0.0017	576 0.0017	28 0.0358
29 0.0346	636 0.0016	612 0.0016	31 0.0323
32.5 0.0308	684 0.0015	642 0.0016	35.5 0.0282

(4A)

?	Ca^{++}	Mg^{++}	K^{+}
	14.5 0.069	12.5 0.08	7.5 0.133
	27.5 0.0364	23.5 0.0425	14 0.0715
	37 0.027	32 0.0312	18.5 0.054
	45.5 0.022	40.5 0.0247	23.5 0.0425
	54.5 0.0184	48 0.0208	28.5 0.0351
	65 0.0154	54 0.0185	32.5 0.0304
	72 0.0139	63 0.0159	36.5 0.0274
	78 0.0124	71 0.0141	41.5 0.0242
	82.5 0.0121	76 0.0132	44 0.0227
	89.5 0.0112	81 0.0124	47.5 0.021

~~ANS 316~~ 67 μMoles ANS - Protein changed

(1)

Protein μg/ml	[Protein]$^{-1}$ (mg/ml)$^{-1}$	ΔF	ΔF^{-1}	ΔF	ΔF^{-1}	ΔF	ΔF^{-1}	ΔF	ΔF^{-1}
		FREE		Ach		Nat		GABA	
24.51	40.8	2.5	0.4	3.5	0.286	4	0.25	3	0.334
48.07	20.8	6	0.167	7.5	0.133	8.5	0.114	6.5	0.154
70.78	14.12	9	0.111	10.5	0.095	13	0.077	9.5	0.111
92.53	10.81	14	0.0715	14.5	0.069	17	0.059	10	0.01
113.6	8.804	17	0.059	17.5	0.057	21	0.0475	12.5	0.08
134.0	7.463	20	0.05	20.5	0.0487	25.5	0.0392	15.5	0.0645
172.2	5.807	25.5	0.0392	26.5	0.0378	33.5	0.0299	21	0.0475
208.3	4.801	31	0.0323	31.5	0.0318	40.5	0.0247	26	0.0386
241.8	4.136	36	0.0278	37.5	0.0267	46.5	0.0216	31.5	0.0318
273.4	3.654	40	0.025	42.5	0.0236	52.5	0.0191	36	0.0278

(2)

Mgmts addeed

FREE	L-Glu	D. Glu	L. Glutamine
2.5 0.4	78 0.0128	62.5 0.016	3.5 0.286
5 0.2	161.5 0.0062	127.5 0.0079	7.5 0.133
8 0.125	241.5 0.00412	189.5 0.0053	11.5 0.087
11.5 0.087	321.5 0.0031	257.5 0.0039	16 0.0625
14.5 0.069	374.5 0.0027	309.5 0.0032	19.5 0.0512
16.5 0.0605	452.5 0.0022	372.5 0.0027	23 0.0435
22 0.0455	554.5 0.0014	458.5 0.0022	30 0.0332
26.5 0.0378	614.5 0.0016	516.5 0.002	34 0.0294
30.5 0.0328	682.5 0.0015	566.5 0.0018	39 0.0254
36.5 0.0274	754.5 0.0013	646.5 0.0016	44.5 0.0225

Free	L-Asp	D-Asp	L-Asparagine
3.5 0.286	78.5 0.0127	80 0.0125	3.5 0.286
6.5 0.154	183.5 ~~0.0055~~ 0.0055	190 0.0053	6.5 0.154
9.5 0.105	255.5 0.0039	268 0.0038	10 0.10
12.5 0.08	321.5 0.0031	334 0.003	13 0.077
15 0.0667	387.5 0.0026	394 0.0025	15.5 0.0645
18 0.0555	441.5 0.0023	454 0.0022	19 0.0525
23.5 0.0425	519.5 0.0019	526 0.0019	24.5 0.041
28.5 0.0351	591.5 0.0017	592 0.0017	29.5 0.034
33 0.0303	663.5 0.00151	658 0.0015	35 0.0286
37 0.027	705.5 0.0014	700 0.0014	39 0.0256

(4)

?	Ca^{+1}	Mg^{+1}	K^{+}
	9.5 0.105	8 0.125	5 0.2
	20 0.05	19 0.0525	10 0.1
	32 0.0312	24.5 0.041	15 0.0667
	41.5 0.0241	33 0.0303	20 0.05
	50.5 0.0198	41 0.0244	25 0.04
	59 0.0169	46.5 0.0215	29.5 0.0339
	73 0.0137	59.5 0.0167	38 0.0263
	85.5 0.0117	69 0.0145	45 0.0222
	93.5 0.0107	78 0.0128	51 0.0196
	108.5 0.0092	87 0.0115	57 0.0176

5

Free	Cs	Li	Ach.
3 0.333	4.5 0.222	5 0.2	4.5 0.222
6 0.165	9 0.111	10.5 0.0955	8.5 0.118
9.5 0.105	13.5 0.074	15 0.0667	12 0.0835
12 0.0832	18 0.0555	21 0.0475	16 0.0625
17 0.059	23 0.0435	25.5 0.0392	21 0.0475
19.5 0.0512	27 0.037	30.5 0.0328	24.5 0.041
23.5 0.0425	35.5 0.0282	39.5 0.0253	31 0.0322
28.5 0.0351	44.5 0.0225	49.5 0.0202	38.5 0.026
33.5 0.0298	50.5 0.0198	56.5 0.0177	45.5 0.022
38 0.0264	57.5 0.0174	64.5 0.0155	51.5 0.0

Abbreviations

ATP	Adenosine triphosphate
Tris	Tris(hydroxymethyl) amino methane
EDTA	Ethylenediaminetetraacetic acid
SDS	Sodium dodecylsulfate
Gly-gly	Glycyl-glycine
cAMP	Cyclic 3'-5' adenosine monophosphate
NADH	Nicotinamide adenine dinucleotide (reduced)
SDH	Succinate dehydrogenase
TCA	Trichloroacetic acid
ANS	1-Anilino-8-naphthalene sulfonate
TNS	2-P-toluidinyl-6-naphthalene sulfonate

5A

Free	Cs	Li	Ach.
4.5 0.222	7 0.143	7.5 0.133	6.5 0.154
8.5 0.118	12.5 0.08	14 0.0715	11.5 0.087
12 0.0835	16 0.0555	20 0.05	16.5 0.0605
15.5 0.0645	23 0.0435	25.5 0.0392	21 0.0475
18 0.0555	27 0.037	29.5 0.034	24 0.0417
21 0.0475	31 0.0322	34 0.0294	28 0.0358
25.5 0.0392	38 0.0264	41.5 0.0241	33.5 0.0298
30 0.0333	43.5 0.023	47.5 0.0211	39 0.0256
33 0.0304	49.5 0.0205	53.5 0.0187	42.5 0.0235
36.5 0.0274	52.5 0.0191	57 0.0176	45.5 0.022